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The report documents 22 presentations comprising 12 poster sessions at symposium. The symposium focused on the state-of-the-art of sampling and analysis techniques that are appropriate for process measurements in the context of an environmental assessment program. Methods are included for qualitative and quantitative chemical characterization of organic and inorganic species in process and discharge streams and biological assays of environmental samples. The symposium was part of EPA's continuing effort to share recently developed methodology and encourage the interchange of ideas between researchers in government, industry, and academia. Topics included: use of sorbents for sampling, sampling of reactive species, sampling and analysis methodology for coal conversion processes, advanced inorganic analysis techniques, advanced organic analysis techniques, and application of bioassay methods to complex samples.

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PROCEEDINGS

Second Symposium
on
PROCESS MEASUREMENTS FOR ENVIRONMENTAL ASSESSMENT

Philip L. Levins, Judith C. Harris, Karen D. Drewitz

Arthur D. Little, Inc.
Acorn Park
Cambridge, Massachusetts 02140

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Industrial Environmental Research Laboratory
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PREFACE

These Proceedings consist of manuscripts for twenty-two papers and abstracts for twelve poster sessions presented at the Second Symposium on Process Measurements for Environmental Assessment held at the Sheraton-Atlanta Hotel in Atlanta, Georgia on February 25-27, 1980.

The symposium focused on the state-of-the-art of sampling and analysis techniques that are appropriate for process measurements in the context of an environmental assessment program. Methods for qualitative and quantitative chemical characterization of organic and inorganic species in process and discharge streams and biological assays of environmental samples were included. The symposium represents a continuing effort on the part of the Process Measurements Branch of the EPA's Industrial Environmental Research Laboratory at Research Triangle Park to share recently developed methodology and encourage the interchange of ideas among researchers in government, industry and academia. Some specific topics represented include:

- Use of sorbents for sampling
- Sampling of reactive species
- Sampling and analysis methodology for coal conversion processes
- Advanced inorganic analysis techniques
- Advanced organic analysis techniques
- Application of bioassay methods to complex samples

The General Chairman of the symposium was Mr. James A. Dorsey, Chief of the Process Measurements Branch, IERL-RTP. The symposium coordinators, Dr. Philip Levins and Dr. Judith Harris of Arthur D. Little, Inc., wish to express their gratitude to Mr. Dorsey for his active participation in the preparation for this symposium. They would like to acknowledge the contributions of session moderators Peter W. Jones of the Electric Power Research Institute, Michael R. Guerin of Oak Ridge National Laboratory, David L. Stalling of Columbia National Fisheries Laboratory and Larry Johnson of Process Measurements Branch, IERL-RTP. Thanks are due to Ms. Karen Drewitz, Mr. John Piecewicz and Ms. Debra Sorlin of Arthur D. Little, Inc. who provided support services at the symposium.

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THE INTEGRATED APPROACH TO CHEMICAL-BIOLOGICAL ANALYSIS

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ABSTRACT

Environmental and health assessments are increasingly dependent on the results of biological testing. Biological testing, in turn, is becoming increasingly dependent on physical and chemical assistance to achieve reliable and interpretable results. This is partly because of the complex and possibly changing compositions of the samples and partly because little is known about the response of many bioassay systems to complex mixtures.

A currently common interaction between disciplines has been termed the "matrix approach". Contributing groups maintain their disciplinary focus but generate data or materials needed by other groups to reach a mutual programmatic objective. Examples are the USEPA environmental assessment projects and the USEPA/USDOE synfuels research sample repository. A more intimate interaction occurs as the contributing groups focus more completely on the program objective. An example is the combined use of chemical class fractionation and biological testing to identify constituents responsible for biological effects. Further integration occurs as the fundamental issues of delivery and dosimetry are addressed. Systems to deliver smokes to experimental animals for inhalation bioassay and to monitor the exposures, methods for determining the dose achieved, and studies of physical-chemical influences on bioassay responses are examples of current work in this area.

The most effective approach to an integrated chemical-biological program requires that each of the levels of interaction be present and that the collaborating groups give the programmatic objective as much attention as is given to disciplinary concerns.

INTRODUCTION

There are a number of approaches (Table 1) to meeting program objectives. All rely on applying the required scientific disciplines to reach the objective. In general, the task of meeting a program objective becomes more difficult as the number of scientific disciplines involved increases. The nature of the product required and the degree of efficiency with which the objective is reached depend very highly on the nature and degree of interdisciplinary interactions. This is particularly the case for Environment Assessment Programs because they deal with a wide variety of sub-objectives and require the input of very many scientific disciplines.

In the "independent" approach (Table 1), each group functions independently and interacts with other disciplines only when it is somehow beneficial to their own work to do so. The groups focus on peers in their individual disciplines for both guidance and reward. The prime product is generally knowledge or methods that constitute fundamental contributions to their disciplines. It is usually a very inefficient way to reach an applied objective but there is always the chance that an advance of such fundamental importance will be made as to solve the problem entirely or at least greatly reduce the time required for its ultimate solution. The primary limitation is novel ideas, creativity. Funding sufficient for the most sophisticated tools and to attract creative talent is also a limitation.

There are a number of interactive (or "cooperative") approaches. They vary in the degree of interaction and interdependence. Interaction can range from little more than a program coordinator's success in bringing the groups together to share results to an interaction that could classify as an "integrated" approach. The focus ranges from pure disciplinary to pure project orientation. The result tends to be data. As the interaction grows stronger the effort results in "information" (a synthesis of the data) and eventually "knowledge" (an understanding of the data and its implications). The interactive approach tends to be a rather efficient approach to meeting an applied objective. The efficiency achieved usually depends on the talents of the project officer. There is no lack of immediate problems which can be attacked using available tools and knowledge so the current limitation tends to be funding available.

In the "integrated" approach, the programmatic objective (e.g. in-vitro bioassay of complex mixtures, environmental control technology, environmental assessment) becomes the disciplinary objective. There must be either much disciplinary interaction or "total" interaction--to the point where the scientific discipline takes only equal (but not less) importance to the applied discipline. The focus tends to be on the project (applied discipline) and the individual scientific disciplines being drawn upon to address the problem. The result is fundamental knowledge and methods as is the case for the independent approach but much of the contribution is to the applied discipline rather than the fundamental scientific discipline. The integrated approach can be either very inefficient (as when the applied objective is peripheral to the issue) or exceptionally efficient (a highly relevant applied objective). Creativity is an important limiting factor here as it was for the independent approach because both rely on insight to resolve fundamental issues. Funding is somewhat more limiting because many of today's problems can be addressed using currently available tools and knowledge.

Examples of on-going programs related to environmental assessments are presented as indicative of the nature of the products generated at various levels of interaction.

INDEPENDENT-INTERACTIVE APPROACHES

Most USEPA and USDOE site-specific environmental assessments may be in this category. Much of the ultimate integration of results is the responsibility of the project officer or of a disciplinary assessment group included among the contractors. As examples of these activities are covered extensively throughout these proceedings, they will not be discussed further here.

Paraho/SOHIO Matrix Equipment

The Paraho/SOHIO study (Table 2) (2) is an example of a loosely coordinated interaction that relies most heavily on the disciplinary expertise of its participants. The approach has proven exceptionally cost-efficient to its project offices because it draws upon the mutual interests (and the funds) of several agencies.

Samples were provided by the Department of the Navy through Development Engineering, Inc. (DEI), and the Standard Oil Company of Ohio (SOHIO). DEI produced 50,000 barrels of shale oil by the Paraho above-ground retorting process and forwarded the product to SOHIO for refining on behalf of the Department of the Navy. The products are being studied as military fuels and for unforeseen environmental/health effects by the Department of Defense.

Samples provided for the matrix study have been distributed (Table 2) to approximately fifteen different groups around the country expert in various chemical, biological, and ecological analysis areas. Each group is examining the samples as part of its basic research program. The groups publish their results as they see fit and meet at least annually to share experiences and results.

A highly definitive characterization of the samples is thus obtained in exchange for highly relevant samples for fundamental study.

Synfuels Research Materials Facility-Repository

The mechanism to achieve the matrix study involved establishing (1) a central materials "repository" or "facility" to ensure that the investigators were provided with the proper samples and that the sample suppliers were provided with the research findings. While the matrix program is loosely interactive (except within some of the participating institutions), the "facility" must be highly interactive and program-oriented. Its activities extend to providing special services in sampling and characterization and in bioassay preparation. Of special importance, it also seeks to fill gaps in the data base and to follow up significant observations by soliciting the input of other groups in exchange for samples.

Several independent and "matrix" experiments are (7) underway in the general area of synfuels technologies though none are as fortunate to be working with such relevant samples as is the Paraho/SOHIO study. The studies (Table 3) involve a variety of sample types, endpoints, and institutions. Samples are described in the broadest of terms and do not emphasize process names or specific technologies. The reason is that we have no evidence that the samples are suitable for process-specific assessments. Operating conditions at the time of sampling as well as sampling and sample handling conditions are seldom adequately specified to draw process-specific conclusions. The samples currently available are best suited for generic research into the general chemical and biological properties of coal- and shale-derived petroleum substitutes as compared to petroleum. They are especially valuable for intercomparing chemical and biological properties, comparing and developing methods, and as "training" or "validating" materials for new contractors.

As more groups examine a given sample and as the groups interact more closely, the samples can become very precious. This is especially the case for highly interactive programs where the groups seek to compare mutagenicity-teratogenicity-carcinogenicity or to identify carcinogens, or to compare new methods with established methods. A series of five materials is currently being collected and characterized to serve as materials suitable for extended study. They differ from other samples primarily in that 30-50 gallons will be available to draw from and in that they are being stored and periodically monitored for stability with attention to long-term utility. The resulting data base will periodically be published and distributed with the samples.

These materials, the current "matrix" type experiments, and the current "facility" concept are targeted to generic research. They serve to bring the talents of independent research groups to bear on programmatic objectives without jeopardizing their primary disciplinary responsibilities. The samples and facility serve as a "resource" to the scientific community and matrix program. Samples are available to any investigator following approval of the sample supplier.

This is a unique route to an interdisciplinary objective because it does not demand individual initiative for a programmatic interaction.

INTERACTIVE-INTEGRATED APPROACHES

Specific aspects of USEPA and USDOE environmental assessments require highly interactive approaches involving several scientific disciplines. Fundamental advances in delivering test materials to biological systems for bioassay and in determining the dose achieved or causative agents present are most likely when the "integrated" approach is used.

Identifying Mutagenic Constituents of Petroleum Substitutes

The traditional approach to identifying bioactive constituents of complex organic mixtures involves several steps. The starting sample is bioassayed, divided into chemically or physically defined fractions, the fractions are bioassayed, and the process is repeated centering on fractions and subfractions exhibiting the desired biological response. Constituents of the bioactive subfraction are identified, suspect constituents are synthesized, and the synthesized compounds are subjected to bioassay to confirm their importance.

Knowing the determinant constituents identifies compounds or properties worthy of chemical analysis and monitoring. It can also suggest measures which might be taken to reduce the biological activity of the materials. The approach generally provides fundamental information on bioactivity-chemistry interrelationships and almost always yields new methods of chemical and biological analyses.

The approach is designed to identify bioactive constituents but it yields useful secondary information in the process. Chemical class fractionation provides data on the general composition of the samples. This is a semi-preparative scale fractionation (typically 5+ gm of starting sample) designed for bioassay rather than quantitation so recoveries can be low and precision only fair. Trends are still evident. The fractionation can be carried out with quantitation in mind if desired.

Results are summarized (Table 4) for two different petroleum crudes, a crude shale oil, a series of coal-derived products (ASOH-atmospheric still overhead, ASB-atmospheric still bottoms, VSOH-vacuum still overheads, VSB-vacuum still bottoms), and a pair of coal-derived "distillates" that are the same except that one had been hydrotreated with relatively high severity. The procedure yields a "volatiles" fraction, a fraction insoluble in ether, ether soluble acids, insoluble acids, ether soluble bases, insoluble bases, neutral saturates, neutral aromatics, neutral polycyclic aromatics, and a neutral polar fraction. Although the results are crude, they prove very useful for judging the nature of the samples--volatility, intractability, acidity, and aromaticity for example.

The mutagenicities of the individual fractions as determined using the Ames test for bacterial mutagenicity (all with TA-98 and Aroclor 1254 induced S-9 activation) are expressed (Table 5) as the number of revertant colonies per microgram of fraction tested. The greater the number of revertants per microgram, the greater the mutagenicity. As a further benchmark, benzo(a)pyrene exhibits about 50 Rev/μg.

A cursory examination of this data, too, proves useful. We note that the volatile material never exhibits much mutagenicity. The acidic constituents of the oils tend not to be mutagenic. The basic constituents and the polycyclic aromatic neutral constituents, however, are quite often mutagenic. For any given petroleum substitute, the ESB fraction is more mutagenic than the neutral polycyclic aromatic fraction. Some of the ESB fractions rival or exceed the mutagenicity of pure benzo(a)pyrene.

Finally, assuming (10) that the mutagenicity of the starting material is the sum of the mutagenicities of the fractions (adjusted for the weight percentage content of the fractions), one can estimate the total mutagenicities of the samples and the percentage of the total contributed by the individual fractions. Using this approach (3-5) we find (Table 6) the petroleum crudes only very slightly mutagenic, the shale oil to be more mutagenic, and the coal-oils to vary greatly in mutagenicity depending on their volatility and processing.

There are also other important trends. The most unexpected of these is the high contribution to the mutagenicities of the petroleum substitutes by their alkaline constituents. The alkaline and polycyclic aromatic neutral constituents are not only the most active subfractions but they are also the primary contributors to the mutagenicity of the samples as a whole.

These observations constitute important results generated in the course of the primary study which itself focuses on identifying the bioactive constituents. The mutagenicity is found (Figure 1) to reside in the relatively non-volatile material and concentrate in the basic and neutral fractions. The neutral mutagens are (9) aromatic rather than aliphatic and are concentrated in the 3-5 fused ring subfractions. Polycyclic aromatic hydrocarbons (9), neutral nitrogen heterocyclics (13), and neutral polar constituents all exhibit mutagenicity. Both the parent and simply alkylated polycyclics and the multialkylated polycyclics contribute (6) to the mutagenicity. The alkaline mutagens are found (10-12) to be among the more polar and more aromatic constituents and reside mostly in the 2-4 fused ring fractions. More detailed study (11,12) shows the determinant alkaline mutagens to be polycyclic aromatic primary amines although there is also some contribution by the alkaline nitrogen heterocyclics.

More detailed study can be carried out to identify individual constituents at the isomeric level if need be. Alternatively, the same general approach can be applied to other sample types or used with other biological endpoints.

This general approach requires considerably more interaction than does the disciplinary matrix approach because chemical and biological observations dictate subsequent biological and chemical steps.

Delivery and Dosimetry

Complete integration of chemistry and biology tends to occur as the central issues of "delivery" and "dosimetry" are addressed. This is especially so when complex mixtures are involved and when the constituents of interest are organic.

Among the most complex efforts to "deliver" a relevant material for biotesting was that carried out by the smoking and health research community. The object (13) was to develop a way to smoke a cigarette the way people do (but more reproducibly) and route the smoke so that it was inhaled by experimental animals in a form not significantly different from that inhaled by humans. At one point there were some thirty criteria that had to be met by the device.

The work has resulted in systems costing a few thousand dollars and up to expose dogs or rodents, to systems costing more than $100K to simultaneously expose several hundred rodents. In the process, many sophisticated issues in dosimetry, aerosol physics and monitoring, aerosol chemistry, sampling and analysis, and inhalation bioassay were addressed.

An example somewhat more relevant to the subject of this symposium is study of the beeswax pellet implantation model for producing cancer in animals. In this model, the material to be tested is mixed or encased in beeswax and the beeswax pellet is surgically implanted into the animal. When smoke condensate was applied (14) to the lungs of rodents using this technique, it was found that the condensate from only a few cigarettes was sufficient to produce lung tumors.

The model was studied (15) chemically by preparing pellets containing cigarette smoke condensate to which ^{14}C tracers of various compounds were added. Physiological saline was pumped across the pellets and the pellets were taken for analysis of ^{14}C after various times. It was found that some constituents are released very quickly while others are released more slowly. Studies using ^{14}C-BaP or dotriacontane showed that 98% or more of the compound remained in the pellet after as many as 28 days of treatment. The rate of release was found to parallel the hydrophilicity of the individual compound. The more hydrophilic the compound, the more rapidly it is released.

Using this model, the physiological test system is exposed to a constantly changing test material and is never exposed to a material representative of the initial sample. The bioassay model is therefore not applicable to complex mixtures containing a wide variety of both hydrophilic and lipophilic constituents.

We have evidence to suggest that similar factors operate upon subjecting lipophilic samples to in-vitro bioassays as is increasingly done in support of environmental assessments. When crude oils are subjected to the Ames test, the sample is frequently observed to distribute throughout the plate as discrete droplets. "Halos" of discoloration may be observed around many of the droplets suggesting a release of at least some constituents into the test medium. Fundamental equilibrium considerations would suggest that the material released first will be enriched in hydrophilic (e.g. phenolic) constituents. Localized high concentrations of such constituents may be responsible for the "toxicity" frequently reported as interfering in in-vitro bioassays. The inhomogeneous distribution of the sample in the plate and the effect that the droplet size, number, and spatial distribution on ultimate dose could account for much of the difficulty in reproducing the results of the bioassays.

A similar effect is likely to accompany other in-vitro bioassays when applied to complex mixtures. The study of these phenomena require a truly integrated biological-chemical approach, sophisticated experimental design, and methods and instrumentation that may not even exist. The results--interpretable and reproducible bioassays, logical limitations of the bioassays, and fundamental delivery and dosimetry knowledge--may be worth the effort.

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TABLE 1

Disciplinary Approaches to Program Objectives

Characteristic	Approaches Independent	 Interactive	 Integrated
Interaction	None-Little	Little-Much	Much-Total
Focus	Discipline	Discipline-Project	Project-Multidiscipline
Product	Knowledge, Methods	Data-Information-Knowledge	Knowledge, Methods
Efficiency	Poor	Fair-Very Good	Poor-Excellent
Limitation	Creativity Funding	Funding	Funding Creativity

TABLE 2

Paraho/SOHIO Shale Oil Matrix Study

	Participants[a,b]							
Material[b]	Chemical Fractionation	Bacterial Mutagenicity	Drosophila Mutagenicity	Mouse Skin Carcinogenicity	Rodent Toxicity	Pond Ecosystem	Marine Ecosystem	Chemical Analysis
Crude Shale Oil	5	1,2,5	9,10	1,5,8	5	5	-	1,5-7
HDT Shale Oil	5	1,2,5	9	1,5,8	5	5	-	1,5-7
HDT Residue	5	1,5	9	1,5,8	5	5	-	1,5-7
Jet Fuel-Shale	5	1,5	9,10	1,5,8	-	5	-	1,5,6
Diesel Fuel Marine (DFM)	5	1,5	9	1,5,8	-	5	4	1,5-7
Petroleum Jet Fuel	5	1,5	9,10	1,5	-	5	-	1,5
Petroleum DFM	5	1,5	9	8	-	5	4	1,5

[a] 1. DOE-Los Alamos Scientific Laboratory
2. DOE-Lawrence Livermore Laboratory
3. EPA-Research Triangle Park
4. EPA-Gulf Breeze Laboratory
5. DOE/EPA-Oak Ridge National Laboratory
6. API-Union Oil
7. API-Exxon
8. EPA-Kettering Laboratory
9. Brown University
10. University of Texas

[b] Partial listing.

TABLE 3

Synfuels Research Materials Facility Components[a]

Materials[a,b]	Endpoints	Institutions
Coal Liquefaction Crude Product Process Stream Refined Product Solid Residue *Coal Gasification* Aqueous Solid Residue *Coal Combustion* Ash *Shale Liquefaction* Crude Product Solid Residue Refined Product Refinery Residue Aqueous *Petroleum* Crude Oil Refined Product	*Chemistry* BaP, PAH Class Physical *Mutagenicity* Bacterial Drosophila Mammalian *Tumorigenicity* Skin Intra-tracheal *Toxicity* Mammalian Aquatic Marine *Teratogenicity*	Oak Ridge National Lab Los Alamos Scientific Lab Lawrence Livermore Lab Pacific Northwest Lab EPA-Gulf Breeze EPA-Research Triangle Park Univeristy of Texas Brown University University of Tennessee University of Connecticut Exxon Corporation Gulf Oil Union Oil Company Mobil Oil Corporation Kettering Laboratory National Bureau of Standards U.S. Department of Navy Laramie Energy Technology Center Bartlesville Energy Technology Center Pittsburgh Energy Technology Center Electric Power Research Institute Hydrocarbon Research, Inc.

[a] Partial listing.

[b] Contributing Technologies: H-Coal, Solvent Refined Coal, Paraho Shale, Synthoil, COED, Zinc Halide, LETC Simulated In-Situ Shale

TABLE 4

Weight Percent Distribution by Chemical Fraction

Material	Weight Percent										
	V	I	ESA	IA	ESB	IB	N_S	N_{Ar}	N_{Ply}	N_{Pol}	T
Petroleum Crude 5301	7.2	-	0.3	2.2	0.2	0.6	55.7	16.5	9.8	6.2	99
Petroleum Crude 5305	19.6	-	0.2	<0.1	0.6	-	51.0	7.4	3.9	1.1	84
Shale Crude 4601	0.3	-	0.4	0.3	3.3	0.5	55.6	27.4	7.0	2.2	97
Coal ASOH 1312	34.7	-	0.9	-	1.1	0.5	11.9	24.6	0.5	<0.1	75
Coal ASB 1313	0.8	-	0.6	0.1	2.1	-	22.9	55.8	6.9	<0.1	89
Coal VOSH 1314	0.3	-	1.2	0.3	2.6	-	13.9	58.5	19.7	<0.1	97
Coal VSB 1315	-	59.5	0.2	0.8	1.8	9.6	1.3	8.0	18.4	0.3	100
Coal Dist 1601	4.2	-	1.4	0.1	1.5	0.1	12.7	48.9	6.3	0.2	76
Coal Dist HDT/H 1604	12.8	-	<0.1	1.1	0.3	1.0	18.5	42.3	1.4	-	77

V = volatiles
I = ether insoluble
ESA = ether soluble acids
IA = insoluble acids
ESB = ether soluble bases
IB = insoluble bases
N_S = neutral saturates
N_{Ar} = neutral aromatics
N_{Ply} = neutral polycyclic aromatics
N_{Pol} = neutral polar fraction
T = total recovery

TABLE 5

Mutagenicity of Class Fractions

Material	Revertants per Microgram									
	V[a]	I	ESA	IA	ESB	IB	N_S	N_{Ar}	N_{Ply}	N_{Pol}
Petroleum Crude 5301	0	–	0	0	0	0	0	x	x	0
Petroleum Crude 5305	0	–	0	0	0	–	0	x	x	0
Shale Crude 4601	0	–	0	0	8	0.1	x	0	1.6	0
Coal ASOH 1312	0	–	0	0	0	0	0	0	0	0
Coal ASB 1313	0	–	0	0	7	1.7	0	0	0	0
Coal VOSH 1314	0	–	0	0	40	0.1	x	0	16	0
Coal VSB 1315	–	0.2	3	0	300	3	x	0.1	3	0.7
Coal Dist 1601	0	–	0	0	9	0	0	0	4	0
Coal Dist HDT/H 1604	0	–	0	0	0	0	0	0	0	0

[a] See Table 4 for definitions.

"x" = $<$ 0.1

TABLE 6

Mutagenicity of Oils

Material	% Contribution V	I	ESA	IA	ESB	IB	N_S	N_{Ar}	N_{Ply}	N_{Pol}	Total Rev/mg
Petroleum Crude 5301	0	-	0	0	0	0	0	66	33	0	5
Petroleum Crude 5305	0	-	0	0	0	0	0	50	50	0	6
Shale Crude 4601	0	-	0	0	69	<1	2	0	29	0	388
Coal ASOH 1312	0	-	0	0	0	0	0	0	0	0	0
Coal ASB 1313	0	-	0	0	99	1	0	0	0	0	143
Coal VOSH 1314	0	-	0	0	25	0	0	0	75	0	4210
Coal VSB 1315	0	2	0	0	85	4	0	0	9	0	6317
Coal Dist 1601	0	-	0	0	37	0	0	0	63	0	346
Coal Dist HDT/H 1604	0	-	-	0	0	0	0	0	0	0	0

[a] See Table 4 for definitions.

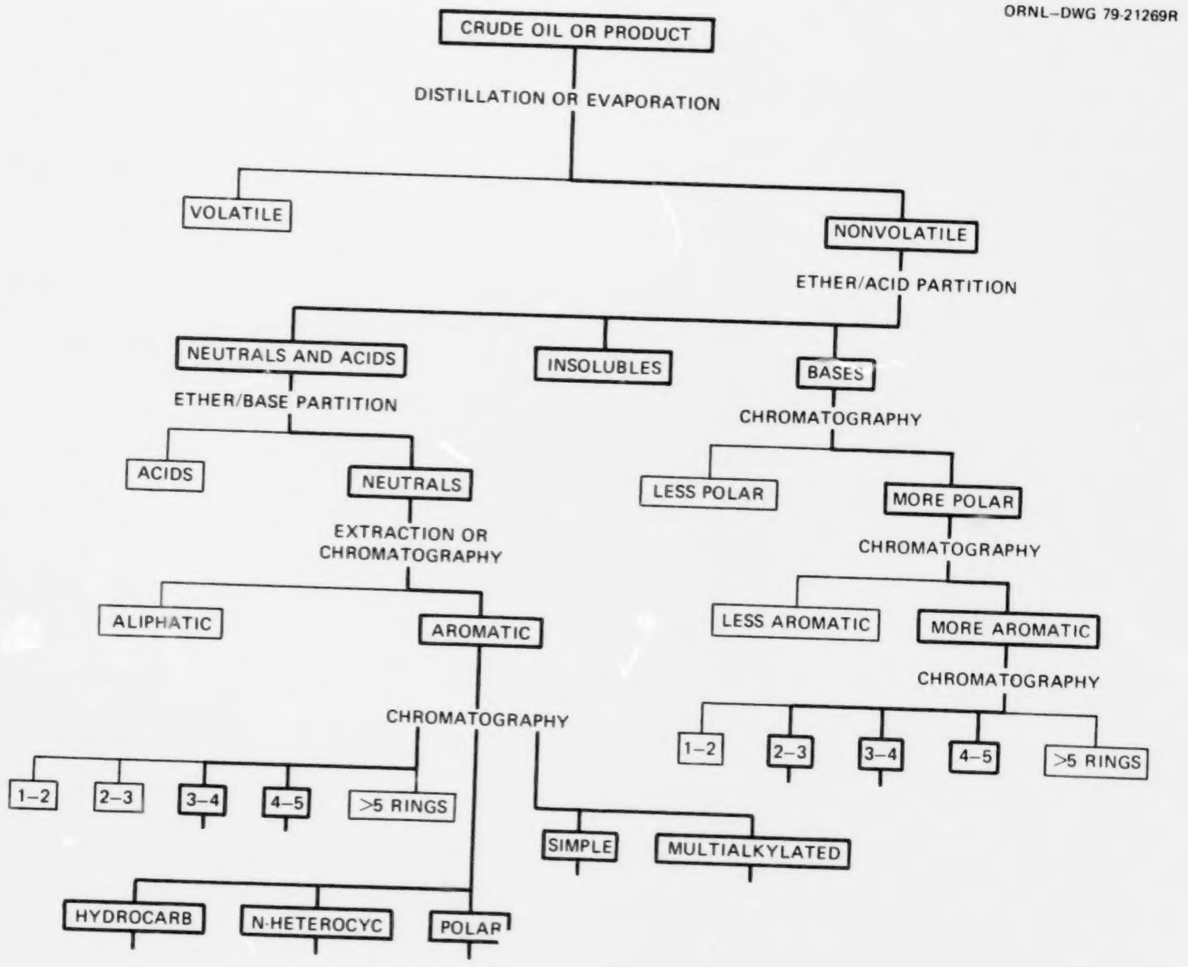
ORNL–DWG 79-21269R
CRUDE OIL OR PRODUCT
DISTILLATION OR EVAPORATION
VOLATILE
NONVOLATILE
ETHER/ACID PARTITION
NEUTRALS AND ACIDS
INSOLUBLES
BASES
ETHER/BASE PARTITION
ACIDS
NEUTRALS
CHROMATOGRAPHY
LESS POLAR
MORE POLAR
EXTRACTION OR CHROMATOGRAPHY
CHROMATOGRAPHY
ALIPHATIC
AROMATIC
LESS AROMATIC
MORE AROMATIC
CHROMATOGRAPHY
CHROMATOGRAPHY
1–2
2–3
3–4
4–5
>5 RINGS
1–2
2–3
3–4
4–5
>5 RINGS
SIMPLE
MULTIALKYLATED
HYDROCARB
N-HETEROCYC
POLAR

FIGURE 1 Subfractionation Path to Mutagenic Constituents of Coal- and Shale-Derived Petroleum Substitutes.

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DESCRIPTION OF BIOASSAY RESULTS AND PROJECTION OF BIOASSAY STUDY NEEDS IN SUPPORT OF A MAJOR SYNFUELS INDUSTRY

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ABSTRACT

Various coals, including peat, lignites, bituminous coals, anthracites, as well as oil shales and tar sands are candidates for conversion to synthetic fuels and/or chemical feedstocks. We have tested the biotoxicity of effluents from a fixed-bed, air-blown, coal gasifier producing 75 ft^3/hr of low BTU gas (200 BTUs/ft^3) from 3 lbs/hr. raw coal, and generating 0.1 lb/hr crude tar effluent.

Four coal types were tested in the gasifier, the crude tars collected, fractionated into chemical classes using a solvent partioning procedure and the fractions analyzed by GC/MS. The crude tars and their chemical fractions were subjected to mutagenicity and cytotoxicity testing using a modification of the (Ames) *Salmonella*/his$^+$ and Chinese Hamster Ovary Cell bioassays, respectively.

The integrated Chemical-Analytical and Biological Testing approach yielded the following information. Whereas the crude tars for certain coal types (Wyoming sub-bituminous and North Dakota Lignite) exhibited borderline or no mutagenicity and cytotoxicity, some of the chemical fractions (organic bases and PNAs) which were present as minority components by percent toal organic mass, exhibited substantial mutagenicity and cytotoxicity. In nearly every case, those crude tars and chemical fractions which were mutagenic, were also cytotoxic, and *vice versa*. Biotesting of crude gasifier tars (and complex organic mixtures in general) may not be sufficient when these mixtures contain highly bioactive minor substituents, or when antagonistic reactions within the mixture mask actual mutagenicity and/or toxicity.

In addition to the specific data presented, this paper summarizes recent progress in sampling, chemical analysis, and bioassay methodology development for synthetic fuel process effluents (complex mixtures). The various streams and effluents of environmental significance are characterized, specifically regarding potentially hazardous (toxic and/or mutagenic) substances. Bioassay tests are specified for various types of samples.

INTRODUCTION

Until about 1910, nearly every major eastern U.S. city had its gashouse, where gas for lighting and cooking was produced from coal by distillation. This formerly widespread technology lost its markets to natural gas and petroleum, but now, with both natural gas and petroleum oil increasingly in short supply, the production of gas from coal is being reexamined as a viable alternative for our energy requirements. Coal is found in 30 of the 50 states, and represents more than 90 percent of the proved reserve of all developed fuels. At the present rate of consumption, if coal were utilized for one-half our present energy requirements, the proved reserve would last about 150 years.

There are more than one hundred different theoretical schemes for coal gasifiers and gas producers (16,7), but none of these will produce a gas product with a heating value equivalent to natural gas (1030 BTU/ft^3). Depending on the process, coal gasification produces a gas product with a heating value from about 125 BTU/ft^3 (low BTU) to about 560 BTU/ft^3 (high BTU). This gas has mainly industrial uses, as synthesis gas for the manufacture of ammonia, plastics, and synthetic fuels, and, when locally produced, as a boiler fuel. This application is especially important since more than 30 percent of the U.S. production of natural gas is consumed by industry (1979 estimate), (12). More important, a high hydrogen-content gas (synthesis gas) produced by coal reaction will be used as the substrate for synthetic liquid fuel (coal liquifaction). High BTU gas which could substitute for natural gas can be produced by methanation of synthesis gas using nickel catalysis. A block diagram illustrating the overall processes involved is shown in Figure 1.

Developments in coal gasification technology must take into account the potential hazard to human health and to the environment presented by fugitive emission and toxic byproducts and effluents. Coal is a complex carbonaceous material, containing sulfur and nitrogen in addition to carbon and oxygen. Theories of coal structure (16) produce models such as that shown in Figure 2. The four most advanced, or most commonly used gasifier processes each produce tars, oil and/or ash and contaminated quench water as environmental effluents, and considering that 10,000 tons or more coal may be reacted per day in a commercial gasifier, as much as 7-8 tons per day of potentially hazardous waste products must be disposed of. Table 1 illustrates the commercial gasifiers and their operating data, including effluent streams.

We report in this study the chemical characterization of and quantification of biotoxicity of effluents from an experimental coal gasifier reacting various coals with a fixed mode of operation, using organic extraction, chemical fractionation and analyses (with gas chromatograph/mass spectra) combined with concurrent short-term *in vitro* toxicity and mutagenicity bioassays.

METHODS

Description of Experimental Coal Gasifier. The effluents tested in our laboratory were produced by a fixed-bed, air blown gasifier producing 75 ft^3/hr of low (200 BTU/ft^3) BTU gas from 3 lbs/hr. raw powdered coal, generating 0.1 lb/hr crude tar effluent, representing 0.3% by mass of the input reactant. The gasifier is so constructed as to permit variable modes of operation (temperature, pressure, air-oxygen mixture, etc.). The reactor is fitted with sampling ports for fugitive emissions and product effluent traps. A flow diagram of the reactor is given in Figure 3. The reaction of four coal types was investigated: Western Kentucky and Illinois (Eastern coals), and Wyoming sub-bituminous and North Dakota lignite (Western coals).

Chemical Extraction and Fractionation. The crude tar effluent collected from the trap of the quench/condensation device is the major effluent stream. The crude tar is extracted with methylene chloride and fractionated by an aqueous acid-base partitioning scheme modified from Novotny et al. (9) by Pellizzari, et al., (10) to yield five organic classes: organic acids, bases, polar neutrals (PN), non-polar neutrals (NPN), and polynuclear aromatic hydrocarbons (PNA). There is usually a 15 percent loss of material during the fractionation. This is a partitioning scheme which is designed more for efficient partitioning and minimal spillover of compounds from one chemical class to another than for maximal recovery. To monitor chemical class overlap, the complex mixtures can be spiked with deuterated standards representative of compounds in each chemical class, and these compounds later identified in gas chromatograph/mass spectra scans of the partitioned classes.

Characterization of the constituents of the five chemical classes, and identification of known toxic substances present was performed by gas chromatograph-mass spectra analysis. A list of representative compounds identified in the chemical classes is shown in Table 2. A summary diagram for this process is given in Figure 4; a detailed description is as follows.

The methylene chloride extract of crude tar was filtered through a Teflon® filter (0.5 μ pore size). The filtrate was evaporated to dryness using a Rotovap®, the solids retained by the filter taken up in 100 ml methanol (B-J), filtered through the Teflon filter, and the filtrate taken to dryness. The filtrates were combined, dried, weighed and redissolved in CH_2Cl_2, the solution spiked with internal standards to allow for quantification and to serve as a quality control parameter for the overall partition/analysis scheme. The standards used were qunioline-d7 (organic base), phenol-d5 (organic acid), dodecane-d26 (non-polar neutral), and anthracene-d10 (PNA).

This solution was extracted twice with equal volumes of 10% H_2SO_4, followed by 20% H_2SO_4. The aqueous phases were combined and washed with CH_2Cl_2, which was combined with the original CH_2Cl_2 phase. The aqueous phase was cooled (ice bath) adjusted with 25% NaOH to pH 10, and extracted three times with CH_2Cl_2 to generate the "organic bases." The aqueous phase was discarded. The original CH_2Cl_2 phase was extracted three times with 5% NaOH, the aqueous phases combined and washed with CH_2Cl_2. The CH_2Cl_2 phase was combined with the original CH_2Cl_2 phase. The NaOH phases were placed in an ice bath and acidified to pH 3 with 20% H_2CO_4, and extracted three-times with CH_2Cl_2 to generate "organic acids." The remaining aqueous phase was discarded.

The original CH_2Cl_2 phase was taken to dryness, reconstituted in cyclohexane, and filtered through a Teflon® filter (0.5 μm). The cyclohexane filtrate was extracted three times with an equal volume of methanol: H_2O (4:1) solution, the methanol:H_2O extract concentrated, and the H_2O removed by freezing-drying to generate "polar neutrals." The cyclohexane phase was extracted three times with an equal volume of nitromethane, the nitromethane phases combined and evaporated to dryness to generate the PNA's." The cyclohexane phase was taken to dryness to generate the "nonpolar neutrals" fraction.

The partition scheme has been validated for efficiency and presence of spillover of chemicals from one class to the other using mixtures of deuterated known substances representative of the individual chemical classes (as described above). Known mutagens were included in the mixture and the recovery of mutagenic activity during fractionation was monitored by bacterial mutagenicity bioassay.

Bioassay

Tissue culture medium and serum was obtained from Gibco Corporation (New York) and KC Biologicals, Kansas City. DMSO was purchased from Fisher Scientific. Bacto-Agar and bacterial nutrient medium was obtained from Difco Corp. NADPH (tetrasodium salt, Type 1) and known positive mutagens (highest purity available) were obtained from Sigma Chemical Company.

Bacterial Mutagenicity. Raw coals, crude reactor tars, and chemical fractions derived from the tars by the partitioning scheme were dissolved or suspended in dimethylsulfoxide (DMSO) and submitted to mutagenicity bioassay using a modification of the Ames/Salmonella bacterial system employing Aroclor-induced rat liver microsomal (metabolic) activation, and bacterial strains TA98 and TA100. Strain TA100 detects base-substitution and strain TA98 detects frame shift mutagens.

Ames Salmonella strains were cultured overnight in Oxoid nutrient broth to stationary phase. The suspension was diluted to an optical density equivalent to 3.3×10^8 cells/ml with saline. For the mutagenicity assay, $\sim 10^8$ cells in 0.3 ml saline were added to 0.3 ml sample, 0.3 ml NADPH or water and 0.3 ml 0.25 M sucrose or rat microsomal activation preparation. For quantitative plate incorporation studies, 2.5 ml molten agar (45°C) was added to the bacteria and sample, the mixture poured onto a 25 ml agar base layer (1.5% Difco Bacto-agar in Vogel-Bonner medium E), and incubated at 37°C for 72 hours. For bacterial toxicity assay, the bacterial suspension was diluted serially in saline to 1000 cells/ml, and 200-600 cells plated in a 2.5 ml molten agar overlay. The assay protocol followed was consistent with the suggestions of DeSerres and Shelby (2), including assay of positive mutagen and solvent controls in each experiment. Triplicate plates were routinely run.

Preparation of S-9 Rat Liver Activation System. The procedure of Ames *et al.* (1) was used. Male Charles River rats, strain CD_1 (200 g) served as the source of liver material. The animals were housed one week before initiation of induction. Food and water were given *ad libitum*. Induced rat liver (minimum of three rats) was obtained from rats injected intraperitoneally on day one with 500 mg/kg of Aroclor 1254 in Corn Oil (0.5 ml of a 200 mg/ml for a 200 g rat). On day five, the animals were sacrificed. The liver was removed, washed twice in cold, 1.15% KCl, blotted dry with sterile paper, weighed, minced, brought to 200 mg/ml protein with 0.25 M sucrose, and homogenized with four strokes of a cold Potter-Elvenhjem apparatus with a Teflon® pestle. The homogenate was centrifuged for ten minutes at 9,000 x g, the lipoprotein layer aspirated off, and the protein concentration of the supernatant adjusted to 30 mg protein/ml with 0.25 M sucrose. The stock solution was checked for sterility, quick-frozen in small aliquots (2-5 ml), and stored for a maximum of two months at -80°C. For the assay, an aliquot of stock solution was slow-thawed and adjusted to an appropriate concentration of protein with 0.25 M sucrose. NADPH was added at 0.32 mg/plate. Each batch was checked against known positive controls using the plate incorporation assay. *S. typhimurium* strains TA98 and TA100 were obtained from Dr. Bruce Ames, (Biochemistry Dept. University of California at Berkeley). NADPH and standard mutagens were obtained from Sigma Chemical Co. (St. Louis).

Chinese Hamster Cell Cytotoxicity. General cytotoxicity was studied *in vitro* using Chinese Hamster Ovary (CHO) cells in tissue culture. CHO cells, clone K1, were obtained from the American Type Culture Association at passage 5, passaged once in our laboratory, frozen in 10% DMSO, and stored at -70°C. Cytotoxicity studies were performed between passage 7 and 15. Cells were maintained in Ham's F12 medium supplemented with 10% calf serum, were passaged at 1:20 dilution (using 0.01% trypsin) and incubated at 37°C in a 5% CO_2-air atmosphere. Cells were split at confluence (about 4 days). For cytotoxicity studies, 10^5 cells were explanted into 35 mm culture dishes (Corning) with 2 ml medium and allowed 24 hours for attachment. The sample was added in 25 µl DMSO, incubated with the cells for 24 hours, the medium was replaced, and the cells incubated further. At 24 hour intervals, the monolayers from triplicate plates were trypsinized and the cells counted. The results were expressed as the fraction inhibition of normal cell growth during the logarithmic phase produced by the toxic material. The effects of raw coals and gasifier tars was compared in each experiment to the growth inhibition produced by a standard cytotoxin, $CdCl_2$ (illustrated by Figure 5). Clonal growth experiments were performed by explanting 200-2000 cells per 35 mm culture dish in 3 µl medium, incubating 24 hrs. for attachment, adding the sample in 25 µl DMSO, incubating 24 hrs for exposure, replacing the medium and incubating 7-10 days. When the colony size reached about 100-200 cells, the medium was discarded, the colonies stained with methylene blue and counted. The results are expressed as fraction reduction of colonies grown in a control medium.

RESULTS

Mutagenicity. The raw coals were non-mutagenic to doses exceeding 10 mg/plate. The crude reactor tars generated from the Western coals (Wyoming subbituminous and North Dakota lignite) were weakly mutagenic; the crude tars from the Eastern coals (Western Kentucky and Illinois) were strongly mutagenic. Mutagenicity in each case required metabolic activation and was confined to frame-shift mutagenesis (active with strain TA98 only). The dose-responsive nature of the mutagenic response and the relative toxicity of the crude tars is shown in Figure 6. Of the chemical classes tested, the organic base was generally the most mutagenic; in some cases generating in excess of 30 revertants/per microgram. The mutagenicity profiles were generally reproducible for separate gasifier reactions of the same coal. This is illustrated by Table 3, which summarizes the crude tar and chemical class mutagenicity for each coal reacted, and by Figure 7. A dose-response mutagenicity profile for the strongly active base fraction is shown in Figure 8. Inspection of Table 3 indicates that for certain coals (Western coals in this case) the crude tar is weakly mutagenic. However, some chemical classes derived from the crude tar, which represent perhaps only a small fraction by mass of the tar, (the base fraction represents ca. 4% of the tar mass) are very strongly mutagenic when tested separately.

Cytotoxicity. The raw coal dusts were non-cytotoxic to doses as high as 10 mg/dish, although the cells seemed to ingest the dust particles, the cell cytoplasm appearing filled with particles after 24 hours. The CHO cytotoxic activity of the crude tars was similar to mutagenic activity. Those crude tar extracts which were strongly mutagenic (Eastern coals) were also cytotoxic, those tar extracts which were weakly mutagenic were also weakly cytotoxic. The basic organic fractions derived from the tar extracts were strongly cytotoxic, and in this regard, the cytotoxicity results were also similar to the mutagenicity results; those fractions which were strongly cytotoxic were also mutagenic. Table 4 summarizes the cytotoxicity data.

DISCUSSION

This study investigated the toxic potential of coal gasification byproducts and effluents. It appears that simple bioassay of crude complex mixtures, such as effluent tar and oils may not provide an accurate estimation of potential health risk. This conclusion is clearly illustrated by inspection of Table 3, where it can be seen that testing of the crude tar generated from the reaction of Western coals would not reveal the potent mutagenicity and cytotoxicity of the organic base fractions. The toxicity of chemical classes representing a small fraction by mass of the crude tar, such as the organic bases, would be masked by the greater mass of non-toxic material present. We conclude that fractionation of such complex organic mixtures into chemical classes be performed, and the fractions then submitted to bioassay. The fact that these toxic materials are present in small mass fractions does not preclude their potential environmental impact, or human health risk, since commercial coal gasification plants reacting 10,000 tons of coal per day will produce about 2-4 tons/day of the strongly toxic and/or mutagenic base fraction alone.

Synthetic fuels systems and effluents having a history of biotesting include fluidized-bed combustion, fixed-bed coal gasification, and shale oil conversion. More toxicological research has been conducted on fugitive emissions and process effluents than on the commercial products themselves. Liquid fuels produced by methanation of product gas have been studied by Epler, _et_ _al_., (3). Synfuels produced from shale-oil conversion by either surface or undergound retorting have been recently studied for their toxic potential, (3). Although end-organ toxicity, (_e.g._, neuro, pulmonary, renal, hepatic, etc.), is certainly of great concern, attention has been given mainly to carcinogenic/mutagenic risk. This emphasis is attributable to the relatively sophisticated bioassay technology available for carcinogenesis/mutagenesis determination.

For complex environmental mixtures, methods for chemical characterization are vastly better developed than for biological testing. Chemical analysis of synthetic fuels, byproducts and effluent streams has been performed in detail (4,13). Analytical chemical analyses include procedures for gas, semivolatiles and volatiles, for fugitive and stack emissions, and chemical extraction and partitioning schemes have been developed for tars and oils (14,6,10,9). Similar acid-base extractive chemical partitioning schemes have been developed for complex organic mixtures such as ambient air and cigarette-smoke particulate (8). More recently, gentler chemical fractionation methods using gel chromatographic procedures for separating non-polar constituents in complex environmental mixtures have been developed for shale-oil (6,4), and have been suggested for other complex organic mixtures to avoid artifact generation by treatment with strong acids and bases. "Class" fractionation procedures all have some overlap of compounds, or "spillover" of certain compounds from one class to another, but experimentation with the fractionation schemes illustrated has minimized this overlap. Chemical identification in all cases has been accomplished with gas chromatographic retention and mass spectra (4,10). Inspection of chemical analytical results helps to identify known mutagenic and/or carcinogenic compounds in complex environmental mixtures.

Epler et al. (3) and Guerin et al. (4) have published evidence that shale-derived oil contained chemical classes which exhibited mutagenicity in bacterial bioassay. Nitrogen-heterocycles appeared to be responsible (3), the organic base fraction having the greatest activity. As for the study reported here, mutagenic activity required metabolic activation and was confined to Ames strain TA98, indicating that frame-shift mutation was predominant. These authors found that chemical class fractions derived from natural petroleum crude oil were very weakly active; less than 0.1 revertant/μg as compared to 30-35 revertants/μg for the same chemical class derived from synfuels from shale-oil or coal gasification. Pelroy and Wilson (11) performed chemical-class fractionation by solvent extraction of high-boiling coal liquids, and the mutagens detectable in the Ames assay system were concentrated in the organic base fractions, which contained nitrogen-heterocycles. Aromatic amines were concentrated in the fractions of a TLC eluate having the greatest mutagenicity in the Ames assay system. Timourian et al. (15) have chemically fractionated effluents and products from underground coal gasification and oil shale processing. Bioassay included mutagenicity in bacteria, toxicity and mutagenicity in different mammalian cultured cells, induction of sister chromatid exchanges (SCE) *in vivo* and *in vitro*, and germ cell toxicity *in vivo*. Again, in this case, organic bases were mutagenic for TA98, although the organic neutrals were also effective mutagens. Crude shale oil is highly mutagenic for bacteria, but only slightly mutagenic for Chinese Hamster Ovary cells. *In vivo*, crude shale oil is negative for SCE induction.

Hsie, et al. (5) have investigated the mutagenicity of the basic (N-heterocycle-containing) fraction of Synfuel A (prepared by the method of Epler, et al.,)(3) in the Chinese Hamster Ovary cell forward mutation system of Hsie, et al. (5). The acetone-extracted ether-soluble base fraction of Synfuel A, which was shown to be the most mutagenic fraction for bacterial bioassay (3) also exhibited the greatest mutagenicity to Chinese Hamster Ovary cells. This acetone-extracted fraction has been shown by Epler et al., (3) to contain higher molecular weight azaarenes, benzacridines and azabenzpyrenes, as compared to weakly mutagenic benzene and isopropanol extracts of the same shale-derived synfuels.

RECOMMENDATIONS

Coal gasification and other forms of synthetic fuels production may well provide at least partial relief for North America's energy problems. But, in light of the results of preliminary toxicity studies environmental and health impact from these industrial processes must not be ignored. This is especially so at a stage of our society when both industrial and population density is high.

We would like to recommend a battery of bioassays to be conducted on synfuels products, byproducts and effluents, including fugitive emissions. Young, *et al*. have described possible occupational hazards associated with coal gasification. These include handling possible wastes and exposure to fugitive emissions, which might include vapor-phase mutagens. The testing battery is designed to be rapid, informative, and cost-efficient, and for this reason will consist of *in vitro* tests. The testing is divided into health and eco-effects, and the battery paradigm is designed for the mode of exposure of the environmental stream. Table 5 illustrates a sample battery testing program which includes mutagenicity, DNA damage (clastogenic effects), and cytotoxicity. *In vitro* battery biotesting is a screening procedure designed to identify potential health risk, but must be substantiated by more complete testing, including skin painting and whole animal carcinogenicity testing, tumor production, and chronic end-organ toxic effects. Other sample battery testing protocols for synthetic fuels byproducts have been suggested, notably by Epler, *et al*., (3). It is further recommended that chemical fractionation and analysis be interfaced with biotesting when environmental complex mixtures are investigated.

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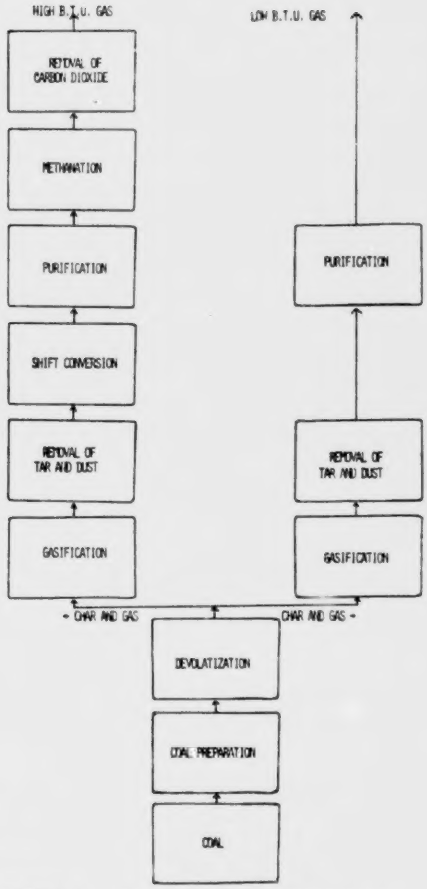
HIGH B.T.U. GAS
LOW B.T.U. GAS
REMOVAL OF CARBON DIOXIDE
METHANATION
PURIFICATION
PURIFICATION
SHIFT CONVERSION
REMOVAL OF TAR AND DUST
REMOVAL OF TAR AND DUST
GASIFICATION
GASIFICATION
← CHAR AND GAS
CHAR AND GAS →
DEVOLATIZATION
COAL PREPARATION
COAL

FIGURE 1 Steps in Coal Gasification

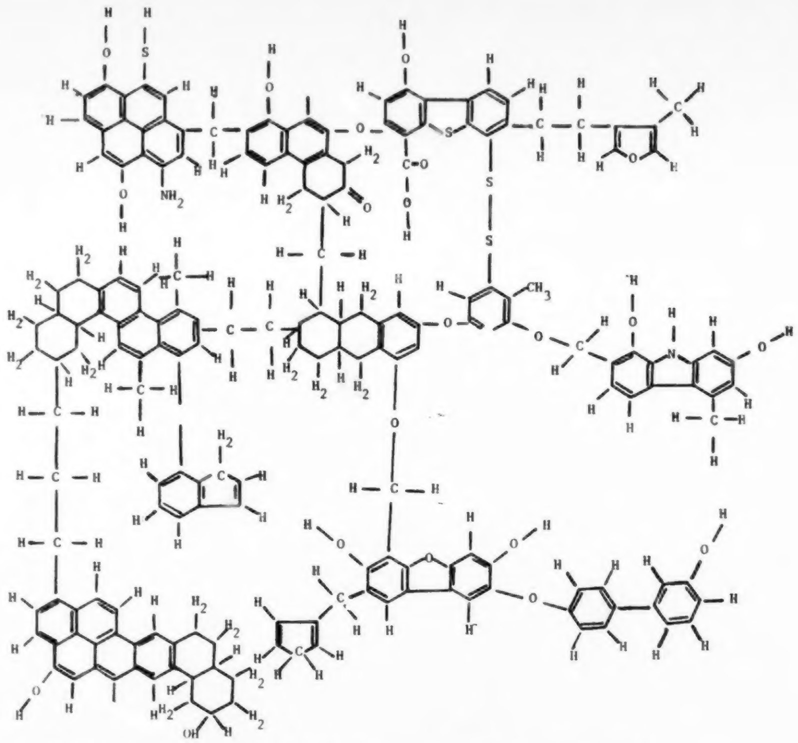

FIGURE 2 Representation of a Hypothetical Coal Molecule.

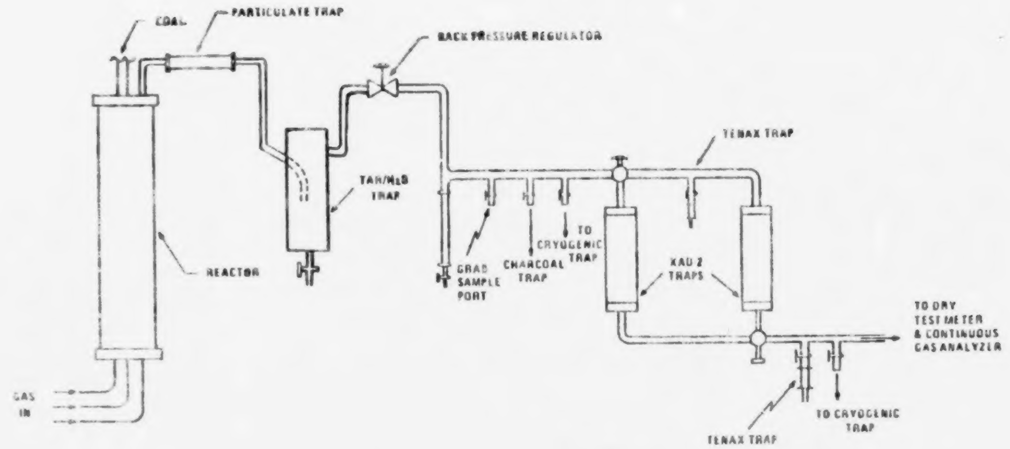
COAL
PARTICULATE TRAP
BACK PRESSURE REGULATOR
TAR/H2O TRAP
REACTOR
GAS IN
GRAB SAMPLE PORT
CHARCOAL TRAP
TO CRYOGENIC TRAP
TENAX TRAP
XAD 2 TRAPS
TO DRY TEST METER & CONTINUOUS GAS ANALYZER
TENAX TRAP
TO CRYOGENIC TRAP

FIGURE 3 Diagram of Experimental Coal Gasification Reactor

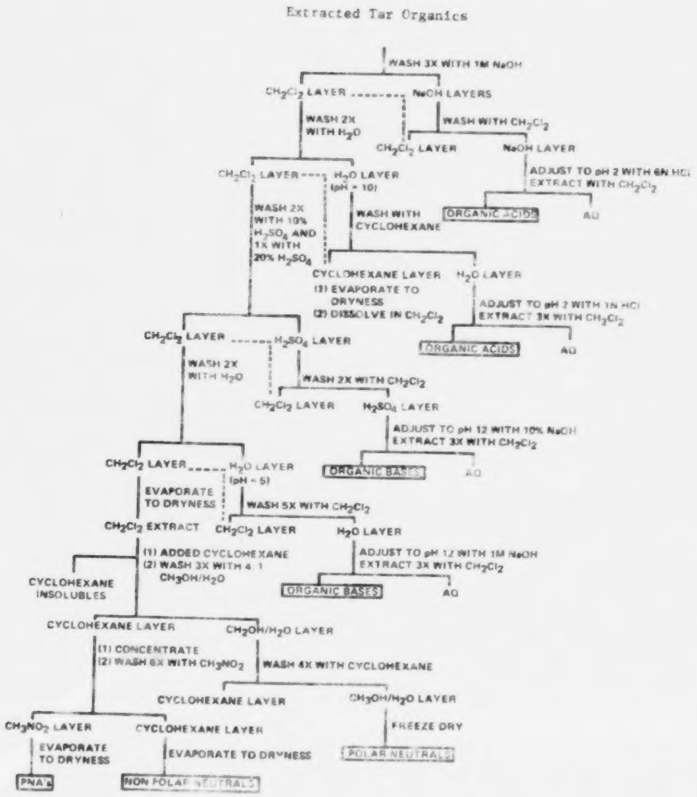
Extracted Tar Organics
WASH 3X WITH 1M NaOH
CH_2Cl_2 LAYER
NaOH LAYERS
WASH 2X WITH H_2O
WASH WITH CH_2Cl_2
CH_2Cl_2 LAYER
NaOH LAYER
ADJUST TO pH 2 WITH 6N HCl
EXTRACT WITH CH_2Cl_2
ORGANIC ACIDS
AQ
CH_2Cl_2 LAYER
H_2O LAYER (pH = 10)
WASH 2X WITH 10% H_2SO_4 AND 1X WITH 20% H_2SO_4
WASH WITH CYCLOHEXANE
CYCLOHEXANE LAYER
(1) EVAPORATE TO DRYNESS
(2) DISSOLVE IN CH_2Cl_2
H_2O LAYER
ADJUST TO pH 2 WITH 1N HCl
EXTRACT 3X WITH CH_2Cl_2
ORGANIC ACIDS
AQ
CH_2Cl_2 LAYER
H_2SO_4 LAYER
WASH 2X WITH H_2O
WASH 2X WITH CH_2Cl_2
CH_2Cl_2 LAYER
H_2SO_4 LAYER
ADJUST TO pH 12 WITH 10% NaOH
EXTRACT 3X WITH CH_2Cl_2
ORGANIC BASES
AQ
CH_2Cl_2 LAYER
H_2O LAYER (pH ~ 5)
EVAPORATE TO DRYNESS
WASH 5X WITH CH_2Cl_2
CH_2Cl_2 EXTRACT
CH_2Cl_2 LAYER
H_2O LAYER
(1) ADDED CYCLOHEXANE
(2) WASH 3X WITH 4:1 CH_3OH/H_2O
ADJUST TO pH 12 WITH 1M NaOH
EXTRACT 3X WITH CH_2Cl_2
CYCLOHEXANE INSOLUBLES
ORGANIC BASES
AQ
CYCLOHEXANE LAYER
CH_3OH/H_2O LAYER
(1) CONCENTRATE
(2) WASH 6X WITH CH_3NO_2
WASH 4X WITH CYCLOHEXANE
CYCLOHEXANE LAYER
CH_3OH/H_2O LAYER
CH_3NO_2 LAYER
CYCLOHEXANE LAYER
FREEZE DRY
EVAPORATE TO DRYNESS
EVAPORATE TO DRYNESS
POLAR NEUTRALS
PNA's
NON POLAR NEUTRALS

FIGURE 4 Fractionation Scheme for Gasifier Tars

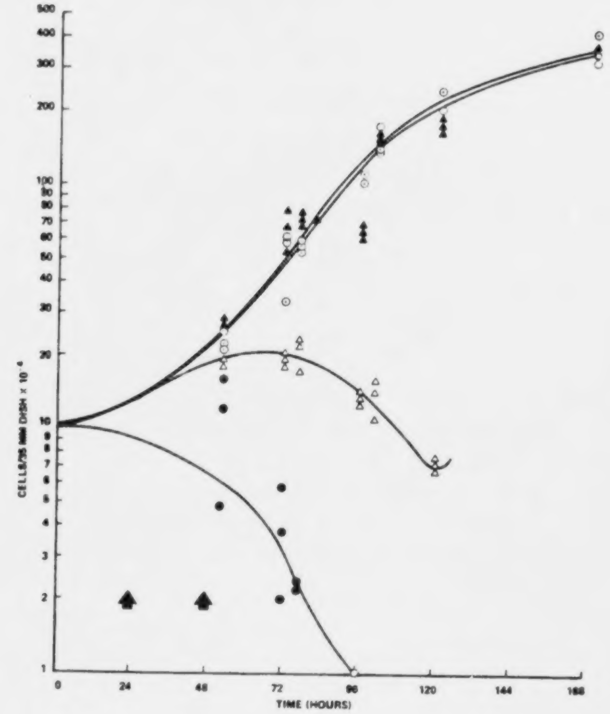

FIGURE 5 Effect of $CdCl_2$ on the Growth of Chinese Hamster Cells in Culture

Triplicate cultures of CHO cells are explanted at 10^5 cells, 35 mm dish, incubated 24 hours at 37°C in a 5% CO_2 atmosphere, and $CdCl_2$ at 10^{-8} M (▲), 10^{-7} M (△), and 10^{-6} M (●), was added in 25 µl DMSO, and the cultures incubated 24 hours (arrows), at which time the medium was replaced with fresh medium and incubation continued, counting cells at 24 hours intervals using an automated cell counter. Control cultures were incubated with 25 µl DMSO (○ , ⊙).

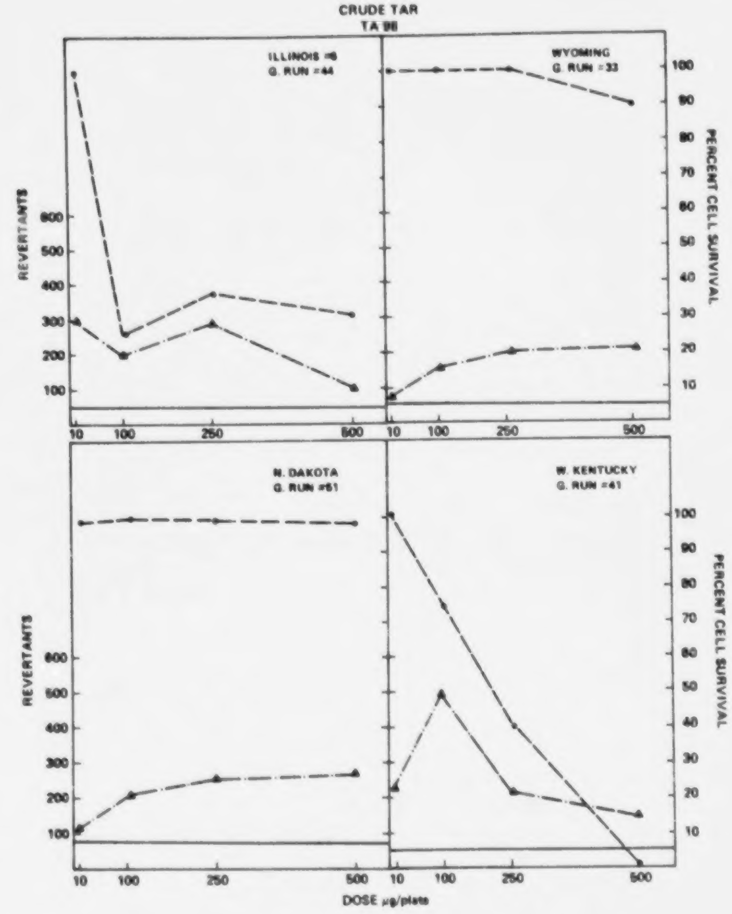

FIGURE 6 Mutagenicity of Four Coal Gasification Crude Tars to Salmonella Strain TA98

Crude tars suspended in DMSO were added at the doses indicated to 10^7-10^8 Salmonella TA98 and 3 mg S-9 microsomal activation preparation, and plated on a histidine deficient agar plate in 2.5 molten agar. For toxicity studies, the bacteria-sample mixture is diluted to 1000 cells/ml and 200 and 600 cells plated in 2.5 molten agar on histidine-containing agar. Mutagenicity results are expressed as total revertants (- ▲ -) uncorrected for toxicity; toxicity results are expressed as percent control cells plated with no sample but including solvent giving rise to colonies on histidine-containing agar (- ● -).

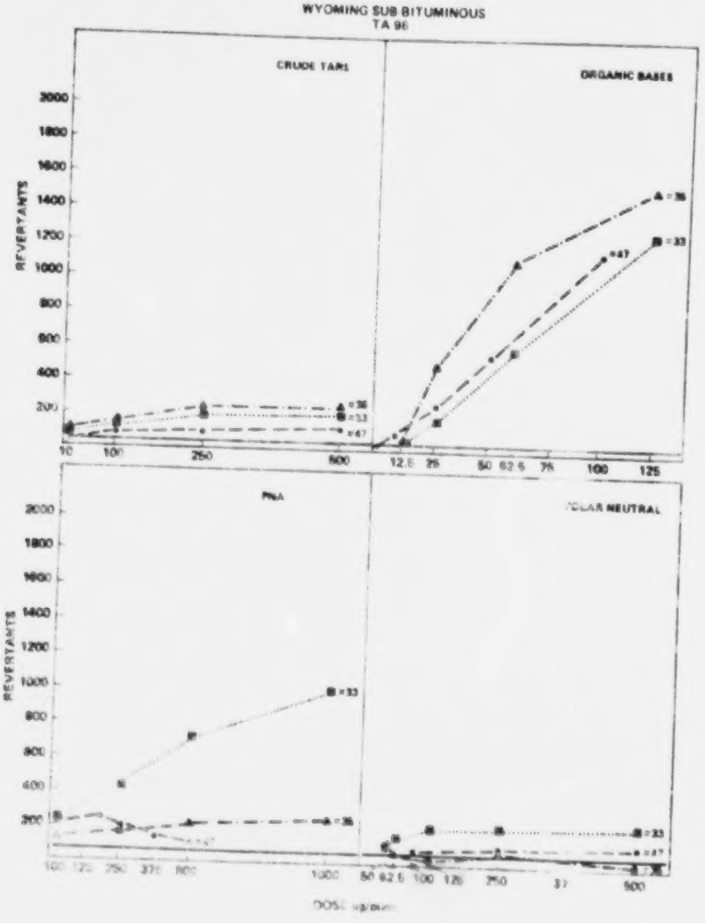

FIGURE 7 Reproducibility of Mutagenicity Determinations for Wyoming Subbituminous Coal Gasification Fractions

The crude tars and chemical fractions from these tars, derived from 3 separate reactions of Wyoming subbituminous coal were assayed for mutagenicity as described in the legend to Figure 6 and the text. Mutagenicity results are expressed as total number of revertants/plate uncorrected for toxicity. Numbers (35, 33, 47) refer to gasifier reaction run.

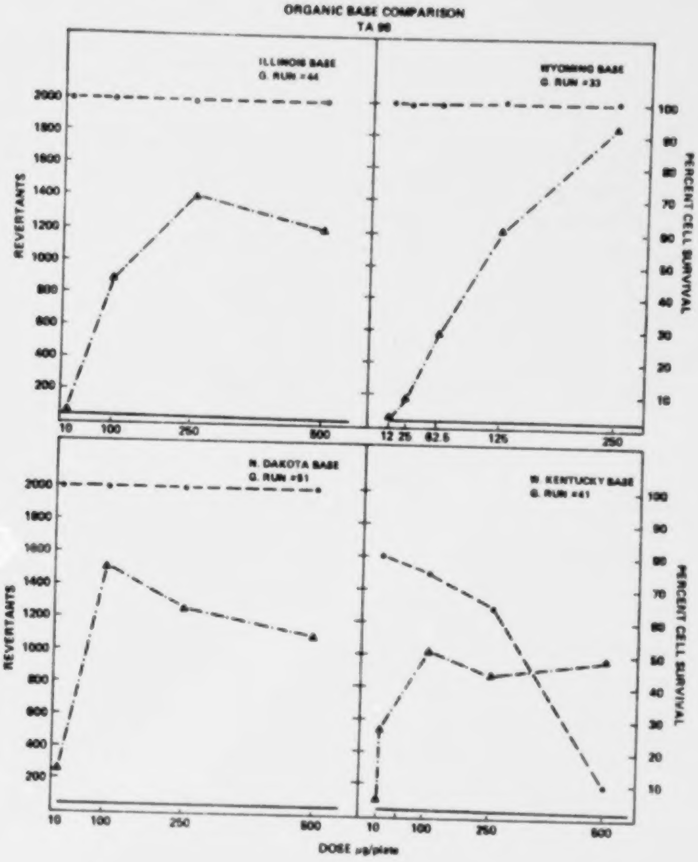

FIGURE 8 Mutagenicity of Organic Base Fraction of Gasification Tars to Salmonella Strain TA98

Organic base fractions partitioned from crude tars of four coal types were tested for mutagenic potential to strain TA98 with 3 mg S-9 microsomal activation preparation, as described in the text and legend to Figure 6. Mutagenicity results are expressed as total revertants per plate (-▲-) uncorrected for toxicity; toxicity results are expressed as percent control cells plated with no sample (but including solvent), giving rise to colonies on histidine-containing agar (- ● -).

TABLE 1

Energy, Tar and Oil Yields From Fixed-Bed Gasification Reactors

REACTOR TYPE (coal type)	GAS PRODUCTION (SCF*/lb)	ENERGY VALUE (BTU**/SCF*)	YIELDS (Percent of Coal Feed)	
			TAR	OIL
LURGI				
lignite	NA***	NA	1.2	4.0
sub-bituminous	35	300	1.5	0.7
bituminous	37	298	3.0	1.0
SLAGGING LURGI				
lignite	NA	NA	1.7	5.6
bituminous	35	375	7.0	NA
WELLMAN-GALUSHA				
sub-bituminous	45	150	3.4	NA
bituminous	65	164	6.0	NA
RILEY-MORGAN				
lignite	∼30	150	3.7	4.0
CHAPMAN-WILPUTTE				
bituminous	∼20	170	10	NA
WOODALL-DUCKMAN				
bituminous	25	200	7.5	NA

* SCF - standard cubic foot
** BTU = British Thermal Unit
*** NA = Data not available

TABLE 2

Quantitative Results for Selected Compounds in Tar Fractions (expressed in μg of compound/gm coal dropped)

Fraction		Run 47 Wyoming	Run 51 North Dakota	Run 52 Illinois No. 6
PNA	Naphthalene	42	384	1,490
	Alkylnaphthalene	44	134	230
	Fluorene	17	59	167
	Dibenzofuran	16	53	191
	Anthracene + Phenanthrene	36	145	801
	Fluoranthene	16	36	276
	Pyrene	15	29	191
	Chrysene	6	28	139
	5-Ring Compounds	Trace	9	112
Organic Bases	Alkylpyridines	18	25	20
	Quinoline	4	25	8
	Alkylquinolines	2	4	8
	Alkylbenzoquinolines	ND	4	111
	Acridine	1	2	2
Organic Acids	Phenol	431	263	15
	Cresols	787	799	32
	Xylenols	954	112	10
	o-isopropylphenol	10	170	<1
	trimethylphenol	86	32	1

GC/MS Conditions: LKB-2091 GC/MS, 15-20 m WCOT capillary column, 1% Se-30/ $BaCO_3$, 100°/2 min/8° min/265°.

TABLE 3

Mutagenicity of Coal Gasification Crude Tars and Fractions

Fraction	Percent of Crude Tar %	Specific Activity[1] (revertants/µg)	Activity[2] (revertants)
North Dakota Lignite			
Raw crude tar	100.0	1.915	191.5
Tar Bases	4.55	17.91	81.49
Tar PNA	36.26	.852	30.89
Tar Polar Neutral	7.76	1.91	14.82
*Tar Acid	23.61	-	127.2^{+}
Nonpolar Neutral	17.1	-	
Wyoming (Subbituminous (33)			
Raw crude tar	100.0	1.189	118.9
Tar Bases	3.33	8.504	28.32
Tar PNA	40.0	1.558	62.32
Tar Polar Neutral	7.50	1.417	10.63
*Tar Acid	26.67	-	101.28^{+}
*Tar Nonpolar Neutral	20.83	-	
Wyoming Subbituminous (35)			
Raw crude tar	100.0	0.399	39.9
Tar Bases	3.44	15.62	53.73
Tar PNA	35.40	.652	23.08
*Tar Polar Neutral	0.28	-	
*Tar Acid	29.55	-	
*Tar Nonpolar Neutral	18.21	-	76.81^{+}
Wyoming Subbituminous (47)			
Raw crude tar	100.0	0.265	26.5
Tar Bases	2.54	5.8	14.73
Tar PNA	36.26	0.36	13.05
Tar Polar Neutral	7.76	0.30	2.33
*Tar Acid	30.70	-	30.11^{+}
*Tar Nonpolar Neutral	27.82	-	
Western Kentucky No. 9			
Raw crude tar	100.0	8.69	869
Tar Bases	6.98	33.79	235.85
Tar PNA	61.79	7.72	477.02
Tar Polar Neutral	5.32	1.766	9.4
*Tar Acid	5.32	-	722.27^{+}
*Tar Nonpolar Neutral	12.62	-	
Illinois No. 6			
Raw crude tar	100.0	11.29	1129
Tar Bases	6.67	6.23	41.55
Tar PNA	66.67	1.965	131.0
Tar Polar Neutral	3.63	37.46	135.98
*Tar Acid	6.67	-	308.53
*Tar Nonpolar Neutral	11.25	-	

---

[1] Specific Activity = (revertants with sample - spontaneous revertants)/dose

[2] Activity = specific activity x fraction mass per 100 µg of composite material (corrected for toxicity)

* Nonmutagenic

+ Additive Mutagenic Activity (fractions comprising the crude tar)

( ) gasification reaction number

TABLE 4

Cytotoxicity of Coal Gasification Tars and Chemical Class Fractions to Chinese Hamster Ovary Cells in Culture.

Sample	% Total Crude Tar	Concentration mg/ml	Mg Sample Per 2 ml	Growth Kinetics % Inhibition	Clonal Efficiency % Inhibition
Western Kentucky					
CRUDE TAR	100.00	10.0	250	83	56
			100	69	45
PNA	61.79	10.0	250	69	23
			100	43	12
POLAR NEUTRALS	5.32	10.0	250	0	0
			100	5	12
ACIDS	5.32	5.0	125	69	12
			50	52	0
BASES	6.98	15.0	375	90	53
			150	74	50
NON-POLAR NEUTRALS	12.62	10.0	250	96	23
			100	90	5
CYCLOHEXANE INSOLUBLES	7.97	10.0	250	22	20
			100	22	17
XAD-2 STEADY STATE	-	5.0	125	79	36
			50	63	38
XAD-2 SURGE	-	5.0	125	22	30
			50	22	9
XAD-2 CONTROL	-	5.0	125	0	26
			50	0	6
H_2O CONDENSATE	-	10.0	250	0	45
			100	0	0
Wyoming Subbituminous					
CRUDE TAR	100.0	5.0	125	36	26
			50	36	5
PNA	36.26	10.0	250	94	80
			100	63	40
POLAR NEUTRALS	7.76	1.0	25	78	38
			10	64	56
ACIDS	30.7	1.0	25	83	54
			10	71	49
BASES	2.54	0.1	2.5	6	12
			1.0	28	10
H_2O CONDENSATE	-	CONCENTRATE	23	45	19
				0	0
Illinois No. 6					
CRUDE TAR	100.0	10.0	250	72	100
			100	72	98
PNA	66.67	10.0	250	77	95
			100	60	70
ACIDS	6.67	5.0	125	0	15
			50	0	18
BASES	6.67	0.1	2.5	0	12
			1.0	0	22
POLAR NEUTRALS	3.63	2.5	62.5	0	42
			25	0	50
NON-POLAR NEUTRALS	11.25	10.0	250	72	88
			100	66	98

TABLE 5

A Sample In Vitro Biotoxicity Screening Battery

Test	Parameter Assayed	Sample Size	Time Required	Route of Administration
Mutagenesis				
Salmonella/his$^+$	(Bacteria) Reverse mutation (point)	100 mg-1 gm	2-4 weeks	variable
Chinese Hamster Ovary Cells (HGRPT)	Forward mutation (point)	100 mg-2 gm	6-8 weeks	variable
Mouse Lymphoma 1.5178Y (TK-1)	Reverse mutation (point)	100 mg-2 gm	6-8 weeks	variable
Salmonella/8-azaquanine	(Bacteria) Forward mutation (point)	100 mg-1 gm	2-4 weeks	variable
Saccharomyces (yeast)	Eukaryotic mutation (point)	100 mg-1 gm	2-4 weeks	variable
Tradescantia	Plant mutation (point) *Detects vapors	10 µg-1 mg	1 week	respirable
Transformation				
C3H 10T.5 cells	oncogenic (Focus-forming) chemical transformation	2-5 gm	12-14 weeks	variable
Syrian Hamster Cell	oncogenic transformation	2-5 gm	12-14 weeks	variable
Enhancement of Viral Transforamtion	oncogenic event *degects inorganic carcinogens	2-5 gm	12-14 weeks	variable
Clastogenic (DNA Damage)				
Sister Chromatid Exchange (Mammalian Cell)	DNA breakage and rejoining	2-5 gm	4-6 weeks	variable
Unscheduled DNA synthesis (Mammalian Cell)	Increased DNA repair activity	2-5 gm	4-6 weeks	variable
Pol A+/Pol A_1 (E. coli)	Increased DNA repair activity (Bacteria)	100 mg-1 gm	2-4 weeks	variable
Cytotoxicity				
Rabbit Alveolar Macrophage	Immunotoxicity to lung	25 mg-1 gm	2 weeks	respirable
Chinese Hamster Ovary Cell	Cytotoxicity	500 µgm-100 mg	3 weeks	variable

PB82-211608

COLLECTION AND RECOVERY OF ORGANICS FROM WATER USING XAD-2 AND XE-347 RESINS

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Arthur D. Little, Inc.

ABSTRACT

Sampling and analysis of organic materials in aqueous industrial effluents has most frequently been approached by procedures using solvent extraction for isolation of the organics. This approach has some well known drawbacks when applied to materials that are highly polar or present at very low concentration. The use of a solid adsorbent material as an in situ extractive sampler appears to be an attractive alternative approach.

A systematic laboratory investigation has been conducted to determine the applicability of macrorecticular resins for general and compound specific sampling of organics in water. The experimental approach involved quantitative characterization of sorbent/sorbate systems by frontal chromatographic analysis of breakthrough and measurement of recovery. Effects of sample flow rate, sorbent type, organic compound type and, to a limited extent, sample matrix have been considered. A mixed resin cartridge, incorporating XAD-2 for collection of non-polar organics and XE-347 for collection of polar organics, has been tested and appears promising for full scale sampling applications.

INTRODUCTION

The qualitative and quantitative analysis of trace levels of organic compounds in aqueous industrial effluents has been the object of increasing interest and study. Current EPA sampling and analysis procedures for organics are directed towards comprehensive characterization, as in Level 1 (9), or towards specific compound quantification as in Level 2 (6) or in priority pollutant analysis (2, 3). Liquid-liquid extraction with an immiscible organic solvent is the approach that has been most widely used for isolation of organics. This approach, which has its roots in the methodology of synthetic organic chemistry, is capable of giving quantitative recoveries of a wide range of non-polar organic materials. On the other hand, transport and extraction of large volume (1-10L) aqueous samples, required to meet detection limit constraints, is cumbersome and frequently complicated by the formation of emulsions. Furthermore, very polar, water-soluble organic species are not always extracted efficiently.

An attractive alternative approach for collection of organics from large quantities of water is the use of an in situ continuous extractive sampler containing a solid sorbent material. An early device of this type, once widely used in the field, was the EPA's carbon adsorption module (CAM) (15) which had generally good collection efficiency, but low recoveries, for organics in water. A number of previous workers had investigated other sorbent media including macroporous resins (7, 8, 13, 14, 16, 17), ion exchange resins (12) and polyurethane foam plugs (10). Based on information available in the literature, it appeared that sorbent approaches offered potential advantages over solvent extraction by a number of criteria:

1. Convenience: A compact sorbent cartridge is more easily handled and shipped than a large volume aqueous sample.

2. Sample Integration: A sorbent approach is inherently compatible with time-integrated sample collection while solvent extraction is more commonly associated with grab or composite/grab aqueous samples.

3. Range of Applicability: Sorbent approaches appear to be suited for ppm and lower levels of organics. Solvent approaches are good for ppm or higher levels.

4. Recovery of polar organics: A sorbent approach can potentially be tailored to allow good recovery of polar organics as well as hydrophobic species.

This paper describes and presents some results of a systematic laboratory investigation of the applicability of macroreticular resins for general and compound specific sampling of organics, based on small scale, chromotographic experiments. The objective was to develop screening procedures and a data base that would allow selection of the resin(s) best suited to a particular sampling application and selection of a sampling module size/configuration that gives quantitative collection of contaminant(s) from the aqueous stream of interest.

EXPERIMENTAL APPROACH

The basic approach used in this work was the generation and analysis of the frontal breakthrough curve produced by challenging a sorbent cartridge with an aqueous solution of organic analyte at constant flow rate. This methodology has been described in some detail elsewhere (4, 5). The approach, which has also been successfully applied to air sampling systems (1, 4, 11), involves determination of the volumetric capacity (V_{50}) of the sorbent for the specific pollutant. The relationship of V_{50} to a characteristic point in the frontal breakthrough curve is illustrated in Figure 1. The volumetric capacity is a quantitative measure of the breakthrough characteristics of the sampling device and is the maximum volume of sample stream that can be pulled through the resin while still retaining the pollutant. The units of V_{50} are mL (of aqueous sample) per cc (of resin bed).

The experimental apparatus employed for initial chromatographic screening studies was a small scale (3.2 cm x 0.5 cm I.d.) sorbent cartridge connected to an LDC UV monitor and Linear Instrument recorder. The cartridge used in the full scale studies was constructed of 15 cm x 3.8 cm i.d. stainless steel tubing. Test solutions were prepared using degassed distilled deionized water and were introduced using a Milton Roy minipump or a Fluidmeter, Inc. Pump.

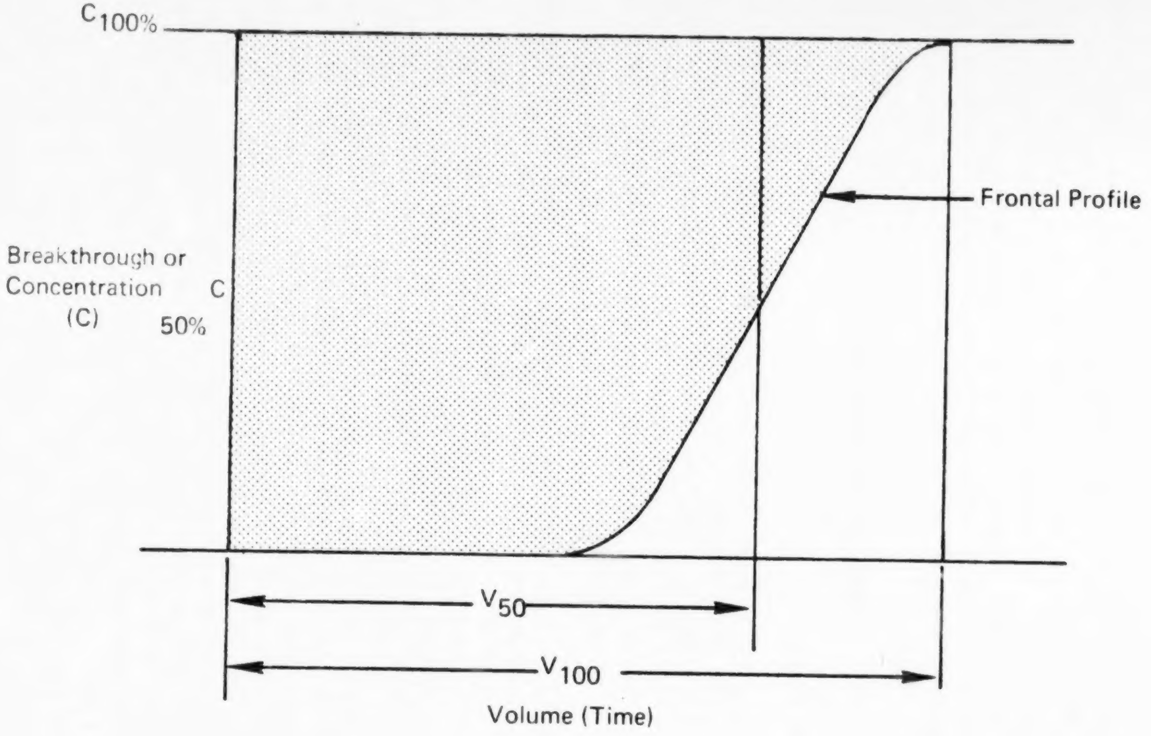
$C_{100\%}$
Breakthrough or Concentration (C)
$C_{50\%}$
Frontal Profile
V_{50}
V_{100}
Volume (Time)

FIGURE 1 Schematic of Frontal Breakthrough Curve Showing V_{50} Volumetric Capacity Point

In the recovery studies, the exposed resins were Soxhlet extracted with methylene chloride or methanol for 24 hours. The solvent extracts were concentrated by Kuderna Danish evaporators to a convenient volume, prior to analysis by GC/FID on a 10% OV-101 column with temperature programming.

The sorbent resins studied were obtained from Rohm & Haas and cleaned by sequential twenty-four hour Soxhlet extractions with distilled, deionized water, methanol and methylene chloride. The cleaned resins were stored in methanol. All other chemicals and solvents used were Reagent Grade. The "real" sample utilized in evaluation of the full scale cartridge was an aqueous effluent associated with a coking facility and generously provided by the facility operator for this research program.

RESULTS AND DISCUSSION

Effect of Sorbent Type

The initial studies in this program were done using XAD-2, the macroreticular resin most extensively previously studied for collection of organic species from water. The results of these studies (5) essentially confirmed the conclusions reported by others: XAD-2 has high volumetric capacity for non-polar organics, but low capacity for species such as phenol or benzoic acid.

Several other macroreticular resins were tested to explore their potential for use as alternatives or supplements to XAD-2 for collection of polar species. These included: XAD-7, an acrylic ester polymer; IRA-904 and IRA-93, anion exchange resins; XE-340, XE-347 and XE-348, carbonaceous adsorbents. The XAD-7 was found to have higher affinity for phenol, but lower affinity for benzoic acid, than XAD-2. This resin was therefore not considered a good candidate for collection of polar organics in a comprehensive sampling system. The ion exchange materials, IRA-904 and IRA-93, were excluded from consideration because it was found that breakthrough on these resins was virtually instantaneous if the aqueous solution contained as much as 250 ppm of sodium chloride. The Ambersorb series of carbonaceous resins, XE-340, XE-347 and XE-348, showed high affinity for a range of polar compounds tested. The volumetric capacity of XE-347 for phenol, benzoic acid and benzyl alcohol at 10 ppm in water is 75 times that of XAD-2. This can be attributed in part to the higher surface area of the XE-347; the XE-347 resin used in this study was found to have a BET surface area of 700 m^2/g versus 330 m^2/g for XAD-2. However, the 75-fold higher V_{50} value found for XE-347 almost certainly reflects stronger specific interactions between resin and sorbate(s) as well. Within the XE series, the V_{50} values were generally comparable and decreased in the order: XE-347, XE-348, XE-340. Based on these resin screening studies, XE-347 was selected as the most attractive candidate to supplement XAD-2 in a general purpose system for collection of organics from water.

Effect of Flow Rate

A series of experiments was performed to determine the effect of flow rate on the V_{50} value. The data in Table 1 show V_{50} values observed for a 10 mg/L acetophenone solution using XAD-2 resin in a small scale (0.5 cm i.d.) cartridge. Mass transfer effects are pronounced at the high face (linear) velocities corresponding to rapid sample flow rates. The net effect of the kinetic non-equilibrium is a decrease in the effective capacity of the column; the V_{50} value at 25 cm/min (6 mL/min) is less than half that at 4 cm/min (1 mL/min).

In sampling applications it is possible to compensate for the decreased efficiency at high flow rate by using a much greater quantity of sorbent than that required for quantitative collection under equilibrium conditions. Such compensation may account for some published reports (e.g., 14) of "no breakthrough" at face velocities of 100 cm/min or higher.

In the limit of low flow rate, <4 cm/min, V_{50} is independent of the face velocity. This information can be used to design a sorbent cartridge that is scaled appropriately for a particular sampling application, as illustrated in Table 2. For example, to collect organics from a 10 L water sample in a 4 hour period (40 mL/min) would require a sorbent cartridge 1.5 inches in diameter or larger. The total quantity of sorbent resin required and thus the necessary depth of the resin bed can be calculated from the sample volume and the V_{50} for the particular sorbent-solute(s) of interest.

Full-Scale, Two-Resin Cartridge

From the earlier screening studies it was apparent that no single resin would suffice to adequately sample a wide range of organic chemical classes. Therefore, a two-stage cartridge with an XAD-2 resin bed upstream of an XE-347 resin bed was designed and constructed. The intent was to allow collection of non-polar, readily adsorbed organics on the XAD-2, from which they could be efficiently recovered. The more polar species, poorly retained by XAD-2, would be trapped by the downstream XE-347 section.

The diameter of the cartridge was set at 3.8 cm (1.5 in) based on a presumed sample size criterion of 10 L in 4 hours as in Level 1 procedures (9). The minimum depth of each resin bed, based on the retention characteristics of the resins used, was calculated to be 3 cm (1.2 in). In order to minimize possible channeling and back eddy effects, however, a bed depth of about 7.6 cm (3 in) was used for each resin. The cartridge is thus, in a sense, overdesigned for this application and could be used for a sample of up to 30 L (12 hr) without breakthrough.

TABLE 1

Sorbent Capacity as a Function of Sample Flow Rate

Flow Rate mL/min	Face Velocity cm/min	V_{50}, mL/cc
6.1	31	60
3.1	16	150
1.6	8.1	280
0.88	4.5	430

TABLE 2

Scale Up of Sorbent Cartridge

Diameter of Resin Bed (in.)	Maximum Flow Rate
1/4	1 mL/min
1	16 mL/min
1.5	40 mL/min
8	1 mL/min

The performance of the cartridge was tested and compared to solvent extraction using both a mixture of 7 model compounds in distilled water solution and a real complex aqueous effluent sample. In each case, the sample was pumped through the cartridge at 40 mL/min. The direction of flow was up (against gravity) through the XAD-2 section and then through the XE-347 section. The effluent was monitored by UV (254) to confirm that <1% breakthrough occurred. The sorbed organics were recovered from the resins either by separate batch extractions of the two sections after removal from the cartridge or by continuous extraction of the intact cartridge, using methylene chloride.

Table 3 presents some of the data for recovery of model compounds via the cartridge and via solvent extraction. The batch extraction data show that the non-polar compounds are collected on the XAD-2 as expected, and do not breakthrough into the XE-347 section. (The low recovery of alkanes such as dodecane has been observed consistently in this work and others (17). It is postulated that this is due to losses from the original aqueous sample onto container walls.)

Phenol, however, is distributed roughly 50:50 between the XAD-2 and the XE-347. Pyrrole, which has even higher water solubility than phenol, is found only in the XE-347 portion of the sampling system.

It was found that methylene chloride and methanol were equally effective at recovery of sorbed phenol from XE-347. Because of this, and because phenol, at least, was distributed in both sections of the cartridge, there was no necessity for or advantage to extracting the two resin sections separately. For convenience, therefore, a continuous extraction procedure applicable to the intact cartridge was adopted. The hardware used was that developed for cleaning XAD-2 resin. (See Reference 9, Appendix D.) Table 3 shows that the total recoveries for batch and continuous extractions of the cartridge are equivalent.

Table 3 also includes data for solvent extraction of a 1 L portion of the model compound test mixture by Level 1 procedures (9). It is clear that the cartridge approach is roughly comparable to, though perhaps slightly less effective than, the solvent approach for recovery of the non-polar organics. (Dodecane is an exception, as noted above.) For phenol and pyrrole, the sorbent approach gives significantly higher recoveries than does solvent extraction.

When the sorbent cartridge was challenged with a real complex aqueous effluent (a coking plant waste), the results were entirely consistent with expectations based on the model compound studies. Figure 2 shows the chromatograms of the concentrated sample extracts obtained by the XAD-2/XE-347 cartridge (2A) and by solvent extraction (2B). The XAD-2/XE-347 extract clearly contains more of the shorter retention time--presumably lower molecular weight, more water soluble--species than does the direct liquid-liquid extract. The peak marked with an asterisk in Figure 2A, for example, which is probably due to phenol (based on retention time and sample source) is only about one-tenth as intense in the

TABLE 3

% Recovery of Model Compounds

Compound	Batch Extraction From XAD-2 Resin	Batch Extraction From XE-347 Resin	Batch Extraction Total	Continuous Extraction	Solvent Extraction
1,2,3-Trichloropropane	73	0	73	71	75
Naphthalene	75	0	75	69	92
Diethylphthalate	78	0	78	86	101
Acridine	91	0	91	-*	89
Phenol	38	46	84	83	17
Dodecane	15	0	15	21	41
Pyrrole	~ 1	64	65	79	46

*Not tested.

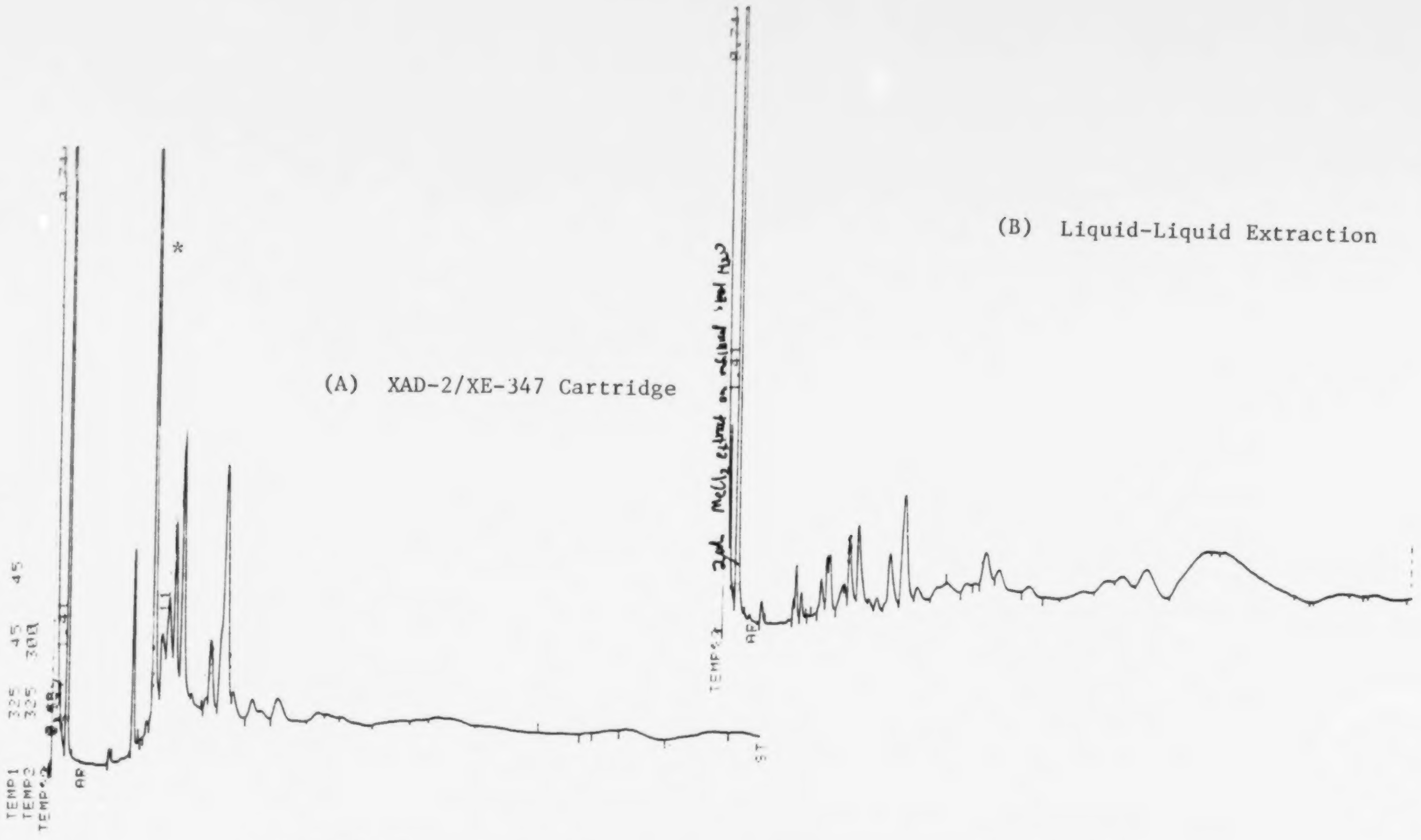

FIGURE 2 Chromatograms of Organics Recovered from Aqueous Effluent Using: (A) XAD-2/XE-347 Cartridge and (B) Liquid-Liquid Extraction (Each injection corresponds to the same aliquot of an original 10L aqueous sample).

solvent extract (2B). The overall recoveries of organics from the aqueous effluent sample by the two approaches are compared in Table 4. The two approaches give equivalent recoveries of the high boiling, high molecular weight (GRAV) range organics. For TCO range, lower boiling, lower molecular weight materials, however, the cartridge approach yields a 50% higher recovery. It is significant that most of the enhanced recovery is apparently due to the types of compounds (e.g., phenol) that are collected on the XE-347 section of the cartridge. However, since this particular sample had a preponderance of GRAV range material, the total recovery for the XAD-2/XE-347 cartridge is only 5% higher than that for the solvent approach.

CONCLUSIONS

The conclusions that have been drawn from the work reported here include the following:

1. Small scale studies have predictive value for sample system design.
2. A slow sample flow rate through the resin is important for quantitative uptake.
3. A combination of resins, XAD-2 for non-polar organics and XE-347 for polar organics, appears to be the most promising general approach.
4. For the XAD-2/XE-347 cartridge, the recovery of most model compounds is comparable to solvent extraction.
5. Phenol and pyrrole recoveries are higher for the cartridge than for the solvent approach.
6. For a real complex aqueous effluent, XAD-2/XE-347 gives better recovery of species such as phenol.
7. For a real complex aqueous effluent, sorbent and solvent give comparable recoveries of GRAV range organics.
8. High levels of suspended particulate in the aqueous effluent will require modifications of sorbent cartridge.

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TABLE 4

Overall Recovery of Organics From Aqueous Effluent Sample

	TCO* mg/L	GRAV** mg/L	Total mg/L
Sorbent Cartridge	8.4	47	55
Solvent Extraction	6.1	46	52

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[*] Total Chromatographable Organics = Materials in about 100°C to 300°C boiling point range.

[**] GRAVimetric Organics = Materials with boiling points above about 300°C.

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APPROACHES TO LEVEL 1 IR AND LRMS MEASUREMENT AND SPECTRAL INTERPRETATION

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ABSTRACT

Approaches to measurement and interpretation of infrared (IR) and low resolution mass spectra (LRMS) taken by four Environmental Protection Agency (EPA) contractors following the guidelines of the Level 1 Environmental Assessment Program were evaluated. The mechanism of evaluation involved supplying each contractor with test spectra and samples for analysis and interpretation. Using IR, the contractors identified 55 to 85 percent of the structural moieties present in the various samples or indicated by the various test spectra, with 85 percent being approximately the maximum attainable. Sources of error in IR measurement included analyst-to-analyst variation in signal location of ±5-10 cm^{-1} and failure to optimize signal intensity through proper loading of the salt plates or KBr pellets. IR interpretation errors disclosed were errors of omission and assignment. To improve IR data quality, all significant signals must be interpreted, including those complementary signals that support or refute other assignments; also, reasonable, alternative interpretations must be considered. The contractors identified 50 to 90 percent of the compound classes represented using LRMS. LRMS interpretation errors disclosed were failure to find molecular ions, reporting molecular ions as fragment ions and vice versa, incorrectly identifying molecular ions and incorrectly assigning ions to homologous series. To improve LRMS data quality, the reference series Eight Peak Index of Mass Spectra should be used, intensity and mass/charge range criteria set to simplify spectra, the LC fraction data used with caution, and IR and LRMS data analyzed independently to avoid bias.

INTRODUCTION

Infrared (IR) and low resolution mass spectrometry (LRMS) are cornerstone techniques in the Level 1 Environmental Assessment program. Infrared spectrometry is used to identify the organic functional groups present in an environmental assessment sample; when sufficient sample is available, LRMS is used in conjunction with the IR technique to determine the principal categories of organic compounds present in these samples. (1)

It is apparent though, from various evaluations of the Level 1 program that these techniques are not always being used by environmental assessment contractors to maximize the output of useful data. To determine the source of contractors' problems with IR and LRMS techniques, a study was undertaken with the following objectives:

- To identify IR and LRMS measurement techniques that either promote or deter the collection of accurate analysis results.

- To identify IR and LRMS spectral interpretation techniques that either promote or deter the collection of accurate analysis results.

- To determine the importance of intangibles such as experience to the collection of accurate analysis results.

The study was conducted in two stages. In the first stage, six IR and six LRMS test spectra were sent to four Environmental Protection Agency (EPA) contractors for interpretation. These spectra were prepared with six samples that varied both in complexity and functional group content. The samples were to represent Level 1 liquid chromatographic (LC) fractions 4, 5, and 6 and were prepared using compounds chosen on the basis of categories expected in these fractions and compounds reported in actual environmental assessment studies. The compounds are shown in Table 1.

The contractors were requested to interpret the spectra as normal Level 1 spectra; i.e., no special efforts were to be made with these spectra beyond that expected for Level 1 work. However, the contractors were asked to clearly indicate the spectral characteristics leading to the identification of functional groups, structural characteristics, or, when possible, individual compounds or compound classes. Using the analysis sheets provided, the analyst was to spell out clearly the reasoning behind each assignment, including information about signal (i.e., peak) wavelength or mass, signal magnitude or shape, patterns of signals, or correlation with LC fraction number.

The test spectra interpretation results returned by the contractors were studied with respect to accuracy, thoroughness of interpretation, approach to interpretation, etc. On the basis of these results, two synthetic samples were prepared and sent to the contractors for both IR and LRMS analysis. The samples for this second stage of the investigation were prepared to represent LC fractions 5 and 6. They were prepared principally from compounds that were used to prepare the stage one spectra and that proved difficult to identify previously. This was done, in part, to determine if measurement of their own spectra would improve the contractors' abilities to make accurate analyses. Included with the two synthetic mixtures was a sample of coal tar from a coal gasification unit. This sample was to be separated using the Level 1 LC scheme, and fractions 4, 5, and 6 were to be analyzed using IR and LRMS. The composition of the two synthetic mixtures and a partial listing of the contents of the tar sample are presented in Table 2.

RESULTS AND DISCUSSION

The IR and LRMS results returned by the contractors were studied with regard to accuracy and sources of error. Approaches to spectral interpretation were investigated through personal visits and/or telephone conversations with the contractor spectroscopists.

IR RESULTS

The accuracy of the analysis results was measured in terms of the percentage of structures in the test mixtures correctly identified by the contractors. These percentages are shown in Table 3. An accuracy level of 80 percent is considered very good; in general, identification at this accuracy level means that only the structures most difficult to identify have been missed. Contractors A and C achieved 60-percent levels on two sample types principally because of low quality spectral data. Contractors B and D achieved low levels principally because of inaccurate and incomplete spectral interpretations. Details regarding these sources of error are presented in the following sections.

IR MEASUREMENT

Several factors of IR measurement that affect final interpretation results were observed. One of these was variation in signal location from contractor to contractor for signals from the same structural source. Such variation was in the range of ±5 to ±10 cm^{-1}. A second factor was failure to optimize signal intensity. Weak signals cannot always be optimized because of insufficient sample quantity; however, overloading of the salt plate or KBr pellet, which can and must be controlled, was observed and had the effect of weak or medium signals being falsely identified as medium or strong signals. A more serious factor observed was the weakness or absence of signals in the 4000 cm^{-1} to 2000 cm^{-1} range in spectra obtained with KBr pellets compared to those obtained with salt plates. This may be due to interaction with the KBr; it may also be due to loss of volatile compounds during the pellet preparation process. This particular factor needs further investigation.

IR INTERPRETATION

Sources of difficulty in IR interpretation include interpreting signals incorrectly, making incorrect though reasonable interpretations, overlooking important signals, and not using complementary signals that, taken together, support or refute identification of a particular structure.

Examples of assignment errors are shown in Table 4. Some of these errors may be subject to debate, though the assignments for signals at 1805 cm^{-1}, 1600 cm^{-1}, 1245 cm^{-1}, and 3052 cm^{-1} clearly seem in error.

Erroneous assignments that are, nevertheless, reasonable occur fairly often. These result from the simple fact that there are different structures giving rise to signals at very nearly the same wavenumber. Examples of such errors are shown in Table 5. Esters, ketones, aldehydes, and acids all have carbonyl signals at about 1700 cm^{-1} and they are often difficult to differentiate, especially in mixtures. A large number of structures such as C-O, C-N, C-C(=O)-C, and C-O-C give rise to signals in the 1100 cm^{-1} to 1400 cm^{-1} range and these, too, are often difficult to differentiate. These erroneous but reasonable assignments arise from sources such as the disagreement among the contractor spectroscopists and the reference works they use for ranges of signals arising from particular structures. This disagreement is illustrated in Table 6.

The nature of the spectra may also contribute to erroneous assignments. Figure 1 compares the spectra from synthetic mixture LC6 (see Table 2), which contained six compounds, and the spectra from the LC fraction 6 of the coal tar. The envelope of signals between 1800 cm^{-1} and 1000 cm^{-1} is not atypical for a real-world sample. The broad, overlapping signals indicate the presence of a variety of different compounds having the same functional groups and/or a considerable amount of intermolecular bonding, e.g., H-bonding. One can readily see that accurate signal assignment with the tar sample spectrum will be difficult.

Errors in signal assignment were observed. However, the majority of errors noted were apparently errors of omission; that is, not making optimum use of the available data. Table 7 shows examples of signals simply not assigned. Another error of omission observed is failure to make optimum use of supplementary data. Table 8 shows examples of such errors. A signal at 1590 cm^{-1}, for example, was described as being due to the NH of an amine. Signals at 1235 cm^{-1} and 1180 cm^{-1}, which could be due to C-N of the amine and thus support the 1590 cm^{-1} assignment, were not described as such. In another case, a signal at 1240 cm^{-1} was described as being due to the C-O-C structure of an ester. The spectroscopist did not note, however, the absence of an ester carbonyl signal at the expected wavenumber value of 1735 cm^{-1}, which would refute the 1240 cm^{-1} assignment.

ADDITIONAL FACTORS AFFECTING IR ANALYSIS ACCURACY

Factors other than errors in assignment and errors of omission that affect analysis accuracy include approach to interpretation, time given to measurement and interpretation, and experience of the spectroscopist.

All spectroscopists approached interpretation in similar ways. They relied on memory to identify most of the major signals, and then used reference texts and tables to identify the minor signals and their possible relationships to the major signals. The time given to interpretation of the spectra dealt with in this study ranged from 1 to 4 hours per spectrum, though the 4 hours included time for assignment of all IR signals to compounds already identified by LRMS. The spectroscopists involved in this study had levels of interpretation experience ranging from 6 months to 25 years. Upon examining these factors and the accuracy levels attained, no strong correlation between accuracy of interpretation and time given to interpretation was found. There was a correlation between accuracy of interpretation and experience of the spectroscopists, though this correlation was also weak.

The accuracy levels attained by spectroscopists with 25 years, 22 years, 11 years, 7 years, 3 years, and 6 months experience (one contractor had three spectroscopists involved in the study) were in the relative order 1, 3, 4, 2, 6, and 5, respectively.

Upon examining all factors affecting accuracy of the IR analyses, it appears that thoroughness of interpretation is most important. In order to achieve a high level of accuracy, the experienced, qualified spectroscopist should (1) interpret all signals of significant magnitude; (2) search out and identify complementary signals that support or refute other signal interpretations, and (3) make alternative interpretations of signals when such interpretations are reasonable.

LRMS RESULTS

The interpretation of Level 1 low resolution mass spectrometry was first studied by supplying each of the four contractors with six low resolution mass spectra obtained with a heated probe sample inlet. Each of the mixtures used to generate the test spectra was represented by a single spectrum like the spectrum for LC6 shown in Figure 2. These spectra were taken under conditions that would optimize as many of the mixture component signals as possible in a single spectrum. In the spectrum shown in Figure 2, the molecular ions for each of the five components are indicated. In the actual test spectra, though, no peaks were singled out. The axes in Figure 2 show that the percent intensity of each ion relative to the most intense ion, 224 (fluorenone carboxylic acid), is plotted vs. the mass to charge ratio as is typical in data-system-generated spectra. One contractor noted that, in their laboratory, a series of spectra are usually taken over varying probe temperatures and at both 70 eV and 15 eV in order to fully characterize a sample. All of the test spectra were taken at 70 eV and only a single probe scan was given in each case. Thus, for this contractor, the test spectra omitted a substantial amount of information.

The contractors' rationale behind the assignment of individual compounds was the focus of both this phase of the study and the test mixture phase. Although one of the goals of Level 1 is to identify categories of compounds as well as individual compounds, the importance of the contractors' category assignments in this study was deemphasized for two reasons. First, although the correct category assignment was made in several instances, it was based on an incorrect compound identification. This could be somewhat misleading to the investigator interested in evaluating the approach to interpretation being taken. Second, in general, Level 1 category assignments tend to be based on the individual compounds identified.

Some of the results of the contractors' analyses of the test spectra are shown in Tables 9, 10, and 11. Each table shows the compounds present, their molecular weights, and the contractors' intensity assignments according to Level 1 (either 1, 10, or 100) for those compounds correctly identified. The daggers indicate correct compound assignments for which the contractor did not report the intensity. In general, the assignments of isomers of the actual compounds were considered correct assignments because isomers usually cannot be differentiated by LRMS alone. For example, ethyl anthracene and dimethyl anthracene were acceptable assignments for 9,10-dimethyl anthracene. The results for test spectrum LC4b (Table 9), a mixture of alkylated anthracenes and naphthalenes, show that 22 out of 24 possible correct identifications were made (91 percent correct). Anthracenes and naphthalenes are probably easily identified in mixtures because most analysts are familiar with their spectra and because these compounds have intense, easily recognized molecular ions. The results for spectrum LC5a (Table 10), a mixture of indoles, carbazoles, and fluorenes, indicate that several contractors had difficulty identifying the carbazoles and fluorenes. The results show that 12 out of 24 possible correct identifications were made (50 percent correct). The results for spectrum LC6b (Table 11), a mixture of a carboxylic acid (1-fluorene carboxylic acid), keto-acid (9 fluorenone-4-carboxylic acid), ether-acid (o-phenoxybenzoic acid), and a phenol, show that very few of these compounds were identified correctly (5 out of 20; 25 percent correct). Contractors A and D did not identify any compounds correctly.

The results of the contractors' analyses of the test mixtures were somewhat improved over the test spectra results. The results for mixture LC6 (Table 12) show that every contractor identified at least one compound correctly and one contractor, C, identified all of the compounds correctly. In all, 11 out of 24 possible correct identifications were made (45 percent correct). A comparison of categories identified in mixture LC6 vs. the results for spectrum LC6b (Table 13) indicates that only ketones were found more easily in mixture LC6. It is also interesting to note that one of the compounds common to both test spectra and mixtures, fluorenone carboxylic acid, can be listed as either a ketone or an acid. In this study it was listed as an acid.

Unlike the interpretation of the synthetic spectra and mixtures, the contractors' assignments of compounds in the coal gasification tar sample were generally based on molecular ions only, with supportive evidence given by the finding of an alkylated homologous series such as alkyl naphthols. In general, the contractors reported the series rather than a single compound.

LRMS INTERPRETATION ERRORS

Five general types of interpretation errors observed in the results from the test spectra and mixtures are:

1. Failing to find molecular ions.

2. Reporting molecular ions as fragment ions.

3. Reporting fragment ions as molecular ions.

4. Incorrectly identifying a molecular ion.

5. Incorrectly assigning ions to homologous series.

Not finding the molecular ion for a compound invariably resulted in the compound not being identified. This was a problem for contractor B in general and for all contractors in the interpretation of spectrum LC6b. Failure to find molecular ions accounted for almost half the compounds missed in the test spectra and test mixtures. The reporting of molecular ions as fragment ions of other compounds and vice versa was also a significant error observed in the results for certain spectra. For example, fluorene in spectrum LC5a (Table 11) was not correctly identified by any contractor but two contractors reported the molecular ion as a fragment ion for carbazole. In the case of fluorene, the error is understandable because many of the most abundant ions for fluorene, including the molecular ion, were also fragment ions of the more dominant components, carbazole and ethyl carbazole. Also in LC5a, contractors A and B incorrectly identified the m/e 180 fragment ion for ethyl carbazole as the molecular ion for fluorenone, a compound not present in the spectrum. In several cases the molecular ion was identified incorrectly as belonging to another compound. Contractors A and B incorrectly identified the molecular ion for ethyl carbazole in LC5a as acridone. The final kind of error observed was that resulting from the spurious identification of an alkylated homologous series based on the identification of a nucleus molecular ion and the subsequent assignments of ions at the appropriate intervals (14 m/e units) to the series members. Contractors B and D made this error in incorrectly identifying the molecular ion for phenazine and 1,10-phenanthroline in spectrum LC5b as being due to fluorenone. Based on the assumed presence of fluorenone, the molecular ion for anthrone was incorrectly identified as belonging to methyl fluorenone. Clearly, although the presence of a homologous series in a sample could potentially simplify the interpretation, the incorrect identification of such a series can lead to many subsequent errors as illustrated by the previous example.

ADDITIONAL FACTORS AFFECTING LRMS ANALYSIS ACCURACY

Factors other than errors in assignment and errors of omission that affect analysis accuracy include the approach to interpretation taken and the interaction with the IR analyst to produce a composite report on the sample. The experience of the spectroscopist, the time given to measurement and interpretation, and the instrumentation employed appeared to have little bearing on the results. Contractors using analog oscillograph recorders achieved essentially the same quality of results as did those employing digital data recording systems.

Following the evaluation of the results of the test spectra and mixture interpretations, the spectroscopists for each of the four contractors were contacted and asked to describe in detail the steps each normally takes in interpreting spectra. A composite of the major factors listed by the contractors that correlated, either positively or negatively, with the more successful interpretations of the spectra and mixtures was then prepared. A successful interpretation was one in which the majority of the compound and category assignments were correctly made. Some of the significant inputs are listed in Table 14 along with the analysts' evaluation of their utility in interpretation expressed either as a "check mark" for agreement with the input shown or as "no" for disagreement. There was little agreement among the analysts. Of the inputs shown in Table 14, all but number 7 (finds homologous series important) and number 9 (uses LC data to verify and exclude) were positively correlated with successful interpretations. All four analysts look for the molecular and at least a few significant fragment ions that can be correlated with the molecular ion. Several look for the eight most abundant ions for a compound and attempt to match these ions to those in reference spectra or lists in the reference Eight Peak Index of Mass Spectra (2). Several analysts try to account for all peaks above a given intensity and mass/charge value, although this will vary from spectrum to spectrum. Several look at the peak intensity ratios of ions suspected of belonging to the same compound to see if these ratios agree with those given in the reference listing. Several find homologous series very important in making assignments. Until this study, none of the LRMS analysts gave the IR data equal weight in making compound/category assignments. All of the analysts make use of the LC fraction in which a suspected compound elutes to verify the compound's presence, but three out of four of the analysts also use the LC data to exclude the presence of compounds not expected in a particular fraction. However, the actual LC fraction in which a compound elutes can be different than expected. The results of this study showed that the exclusion of compounds based on the LC fraction is not always warranted. Contractor A deleted the listing of a fused nonalternate hydrocarbon (benzofluorene) from the results for spectrum LC5a because the LC fraction was two fractions too late for such compounds. Contractor A also felt that spectra taken at low ionizing voltage were helpful in identifying molecular ions. The feasibility of using low ionizing voltage is, however, dependent on the type of mass spectrometer being used. In general, an examination of the inputs that lead to successful interpretations suggests that a systematic approach and a thorough approach go hand-in-hand.

IR data also can improve the overall quality of the LRMS interpretation. After reviewing the IR interpretation results of the test spectra and samples, the LRMS analysts in many cases revised their observations, often by adding new compounds or categories and by confirming the presence of compounds already listed based on the LRMS alone. Table 15 shows several examples of how the IR data were used to produce a more accurate composite set of results. LRMS analyst A originally and correctly listed anthrone as part of mixture LC5 but when the analyst could not find a reference spectrum of anthrone with which to verify the LRMS observation, he deleted anthrone. A more gratifying result of the IR's contribution occurred when the same LRMS analyst changed the assignment of hydroxyanthraquinone in mixture LC6 to that of the correct assignment, fluorenone carboxylic acid. This, in turn, lead to the analyst finding fluorene carboxylic acid, which was also in the sample. LRMS analysts B and D initially found nothing interpretable in spectrum LC6a and test mixture LC6, respectively, until the IR data were consulted. Then both were able to make correct assignments of pyridone (B) and aromatic acids (D) in these cases. Analyst B also correctly changed the interpretation of LC5a from acridine to carbazole and fluorenone to ethyl carbazole after reviewing the IR results. Analyst C used the IR data to confirm carbazole in LC5a and to determine that anthracene was a major component in LC4a. Finally, analyst D added the categories aromatic acids, esters, and ketones to the results reported by LRMS alone in tar sample LC fraction 6. There were many other examples of how the IR aided the LRMS interpretation. One immediate consequence of this study is that at least one of the contractors is now going to utilize the IR data on a more equal basis with the LRMS data.

RECOMMENDATIONS FOR IMPROVED LRMS DATA QUALITY

Based on the results from the analysis of the test spectra and samples and discussions with the spectroscopists, several recommendations were made to improve the quality of the compound and category assignments made in Level 1 studies. Major recommendations are:

1. The reference series Eight Peak Index of Mass Spectra (2) should be used to interpret and verify spectra. Other references tend to be incomplete and/or inaccurate.

2. Intensity and mass/charge range criteria must be set to ensure a systematic and efficient interpretation.

3. The IR and LRMS analysts should first analyze their data independently and then consult with one another to produce a composite report. The initial independent analyses will prevent biasing of the interpretation.

4. The IR and LRMS data should be used as primary inputs to spectral interpretations. Other inputs, such as the LC fraction, the sample source, and previously generated data on similar sources, should be used with caution as aids in spectral interpretation.

5. Polyfunctional compounds like fluorenone carboxylic acid and phenoxybenzoic acid should be listed in both possible categories to avoid confusion and because both functional groups may be active from a health effects standpoint.

6. Suggestions for utilization of the information collected in this study include the preparation of guidelines for mass spectral and IR interpretation for analysts beginning Level 1 work, sets of worked out examples of spectra, workshops in which analysts can exchange information, and spectral interpretation tests.

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2. Imperial Chemical Industry, Ltd., Eight Peak Index of Mass Spectra, First Edition, Mass Spectrometry Data Center, Alder Maston, Reading, United Kingdom, 1970.

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TABLE 1
Mixtures Used to Prepare Test Spectra*

Mixture LC4a

naphthalene (6.2)
anthracene (37.1)
phenanthrene (6.2)
9-methyl anthracene (25.1)
9,10-dimethyl anthracene (25.4)

Mixture LC5a

fluorene (9.1)
2,3-benzofluorene (4.9)
N-ethyl carbazole (52.1)
2-methyl indole (20.0)
2,3-dimethyl indole (4.9)

Mixture LC6a

2-hydroxypyridine (67.9)
2,6-dimethyl phenol (16.5)
o-o'-biphenol (15.6)

Mixture LC4b

naphthalene (35.0)
anthracene (23.3)
phenanthrene (23.4)
9-methyl anthracene (6.2)
9,10-dimethyl anthracene (6.2)
2,3-dimethyl naphthalene (6.3)

Mixture LC5b

7,8-benzoquinoline (16.1)
phenanthradine (30.5)
1,10-phenanthroline (15.5)
phenazine (30.2)
anthrone (7.7)

Mixture LC6b

9-fluorenone (22.6)
o-phenoxybenzoic acid (22.5)
1-fluorene carboxylic acid (22.2)
9-fluorenone-4-carboxylic acid (21.7)
2,6-dimethyl phenol (11.2)

*Weight percent of each component in mixture shown in parentheses.

TABLE 2
Test Mixture

Mixture LC5

fluorene (4.5)
2,3-benzofluorene (4.1)
7,8-benzoquinoline (19.4)
1,10-phenanthroline (31.0)
phenazine (16.8)
2,6-dimethyl quinoline (16.8)
anthrone (7.5)

Mixture LC6

9-fluorenone (26.1)
1-fluorene carboxylic acid (11.1)
9-fluorenone-4-carboxylic acid (41.5)
2-methyl-1-tetralone (5.2)
2-t-butyl phenol (6.3)
2-methyl-1,4-naphthoquinone (9.8)

Coal Gasification Tar Sample; Compounds Expected in Each Fraction*

LC4

dibenzofuran
carbazole

LC5

overlap from
LC4 and LC6

LC6

benzidine
alkyl pyridines
quinoline
alkyl quinolines
alkyl benzoquinolines
acridine
phenol
cresols
xylenols
o-isopropylphenol
trimethylphenol

*Limited analysis based on GC measurements.

TABLE 3
Accuracy of Structure Identification Using IR*

	Contractor			
	A	B	C	D
Test Spectra	85%	65%	85%	60%
Test Samples	55%	55%	80%	80%
Tar Samples	80%	70%	65%	85%

*All percentages shown have an uncertainty of about ± 10 percent.

TABLE 4
Selected Examples of Errors in Assignment of IR Signals

Signal, cm^{-1}	Apparently Erroneous Assignment	Apparently Correct Assignment
3052	OH	Unsaturated CH
1805	Carbonyl	Overtone or summation band
1600	Unsaturated CH	Aromatic $C=C$
1600	$\Phi C=O$	Aromatic $C=C$
1480, 1470	Unsaturated CH	CH_3
1510	$C-N$	Aromatic $C=C$
1245	$\Phi C=O$	$C-O$ of aromatic alcohol
1190, 1160	$C-O-C$	$C-O$ of alcohol

TABLE 5
Selected Examples of Reasonable, Though Erroneous, Assignments

Signal, cm^{-1}	Erroneous Assignment	Apparently Correct Assignment
1715, 1690	Carbonyl	Overtone or summation bands
1710	$C=O$ of ester	$C=O$ of ketone
1710	$C=O$ of acid	$C=O$ of ketone
1280, 1240	$C-O$ of aromatic alcohol	CH_3
1235	$C-O$ of aromatic alcohol	$C-N$ of carbazole
1300	$C-N$ of aryl amine	$OH/C-O$ coupling
1310	$C-N$ of secondary amine	$C-C(=O)-C$ of ketone

TABLE 6
Wavenumber Ranges Indicated for Different Structures as Found in Three Different Reference Sources

Structure	**Reference Source**		
	N. B. Colthup[a]	**Dow Chemical**[b]	**Nakanishi**[c]
OH bend of alcohol	1460–1260 cm^{-1}	—	1500–1200 cm^{-1}
C – O stretch of alcohol	1290–1000 cm^{-1}	1260–980 cm^{-1}	1200–1000 cm^{-1}
NH bend of primary amine	1640–1560 cm^{-1}	1665–1625 cm^{-1}	1640–1560 cm^{-1}
Aromatic C = C	1650–1550 cm^{-1} 1540–1450 cm^{-1}	1665–1615 cm^{-1} 1515–1370 cm^{-1}	1600–1450 cm^{-1} —
C – O – C of aliphatic ether	1175–1055 cm^{-1}	1140–1090 cm^{-1}	1200–1040 cm^{-1}
C = N stretch	1700–1590 cm^{-1}	1630–1590 cm^{-1}	1690–1640 cm^{-1}

[a] N. B. Colthup, *J. Opt. Soc. Am., 40:* 397, 1950.

[b] IR spectral assignment chart, Dow Chemical Company, Midland, Michigan.

[c] Koji Nakanishi, *Practical Infrared Absorption Spectroscopy,* San Francisco: Holden-Day, Inc., 1962.

TABLE 7
Selected Examples of Unassigned Signals Found in Various Spectra

Signal Not Assigned, cm^{-1}	**RTI Assignment**
2930, 2850	Saturated CH
1680, 1670	Carbonyl
1430, 1240	Coupling of acid OH/C – O groups
1450, 1465, 1390, 1380, 1364	CH_3
1240, 1160, 1100	C – O of alcohol
1015	C – O – C of ether

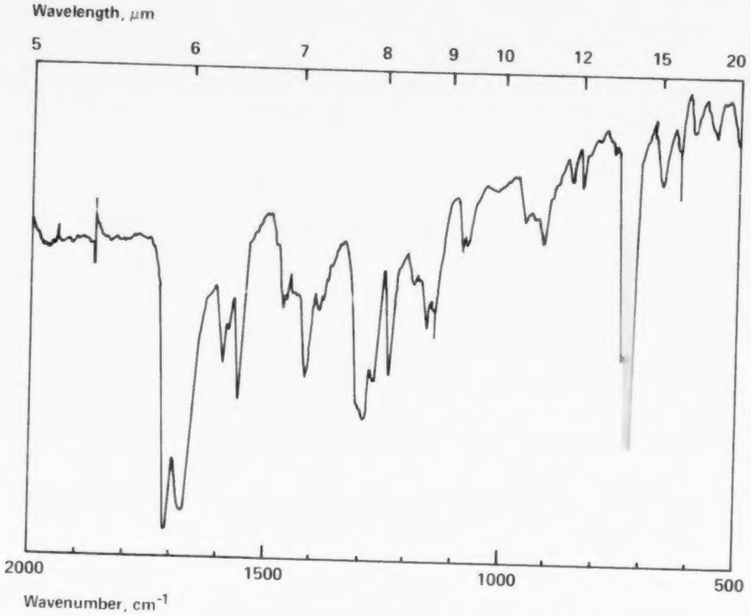

Figure 1a IR spectrum, LC6, synthetic mixture.

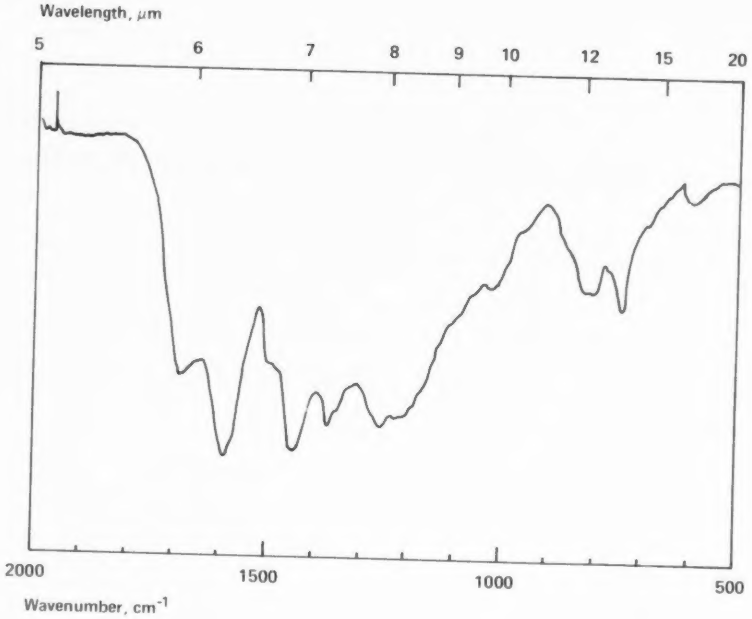

Figure 1b IR spectrum, LC6, coal tar sample.

TABLE 8
Incomplete Utilization of Available Data

Signal, cm^{-1}	Assignment	Complementary Data Not Used
1737	C = O of ester	1160 cm^{-1}, 1100 cm^{-1} (C – O – C of ester)
1690	C = O of acid	3300 cm^{-1} (OH) 1440 cm^{-1} (OH/C – O coupling) 940-920 cm^{-1} (OH bending)
1590	NH of amine	1235 cm^{-1}, 1180 cm^{-1} (C – N of primary amine)
1240	C – O – C of ester	*No signal* at 1735 cm^{-1} (C = O of ester)

TABLE 9
Compounds in Test Spectrum LC4b Correctly Identified by LRMS

Compound*	MW	Weight %	Contractors A	B	C	D
Naphthalene	128	35.0	10	NR	10	100
Anthracene	178	23.3	100	†	100	100
9-Methyl anthracene	192	5.9	10	†	10	100
Phenanthrene	178	23.4	100	†	100	100
9,10-Dimethyl anthracene	206	6.2	10	†	10	10
2,3-Dimethyl naphthalene	156	6.3	10	NR	10	10

*Credit given for assignment of isomers of LC4b compounds.

†Compound reported but intensity not given.

NR = not reported.

TABLE 10
Compounds in Test Spectrum LC5a Correctly Identified by LRMS

Compound	MW	Weight %	Contractors A	B	C	D
Fluorene	166	9.1	NR	NR	NR	NR
2,3-Benzofluorene	216	4.9	NR	NR	10	NR
N-ethyl carbazole	195	52.1	NR	NR	100	100
2-Methyl indole	131	9.0	10	NR	10	10
Carbazole	167	20.0	100	NR	100	100
2,3-Dimethyl indole	145	4.9	10	NR	10	10

NR = not reported.

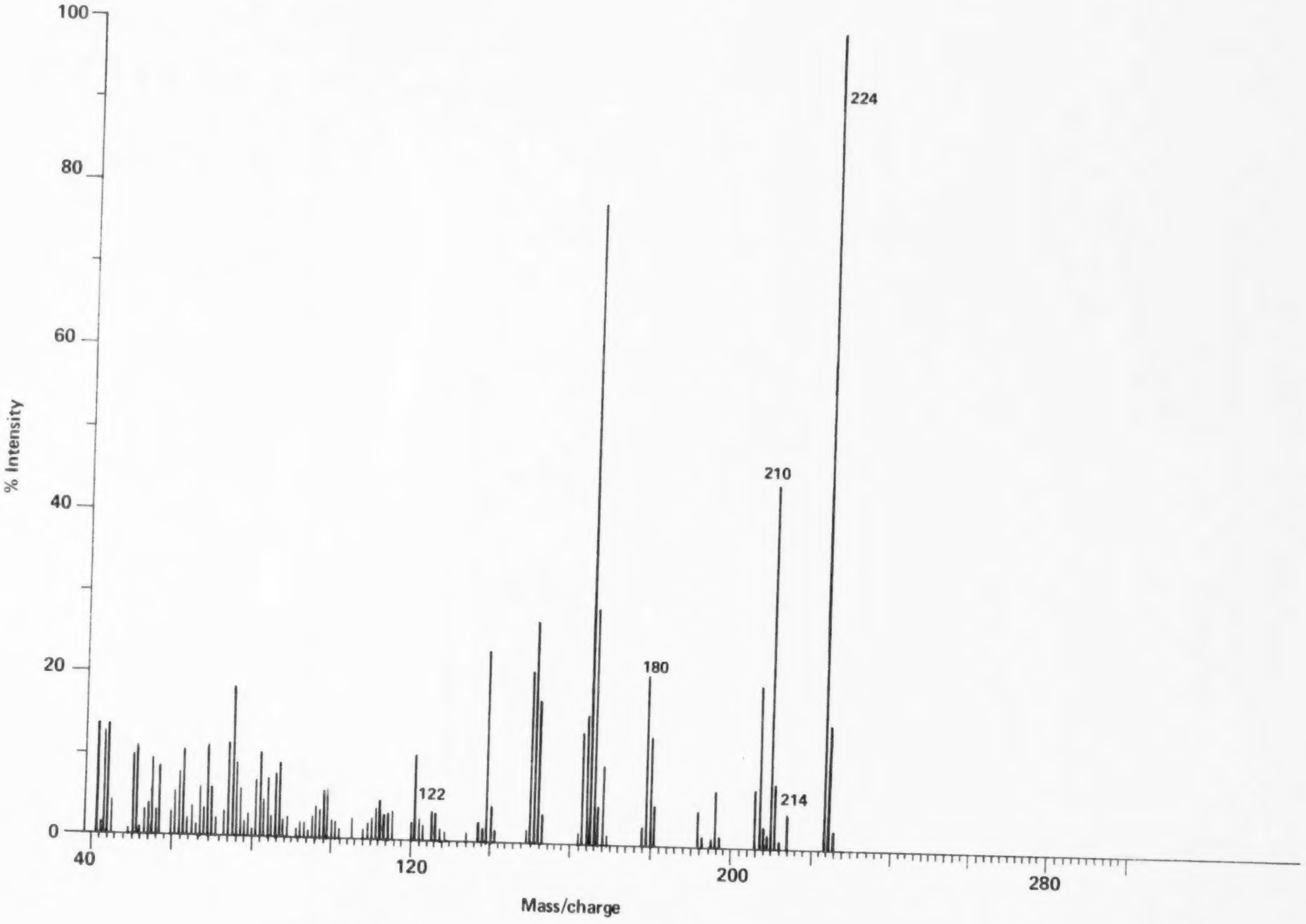

Figure 2 Test spectrum LC6b. Molecular ions are indicated.

TABLE 11
Compounds in Test Spectrum LC6b Correctly Identified by LRMS

Compound	MW	Weight %	Contractors			
			A	B	C	D
9-Fluorenone	180	22.6	NR	NR	NR	NR
2,6-Dimethyl phenol	122	11.2	NR	NR	NR	NR
o-Phenoxy benzoic acid	214	22.5	NR	NR	10	NR
1-Fluorene COOH	210	22.2	NR	†	10	NR
9-Fluorenone-4-COOH	224	21.7	NR	†	100	NR

†Compound reported but intensity not given.
NR = not reported.

TABLE 12
Compounds in Test Mixture LC6 Correctly Identified by LRMS

Compound	MW	Weight %	Contractors			
			A	B	C	D
9-Fluorenone	180	26.1	100	100	10	10
1-Fluorene COOH	210	11.1	NR	NR	10	NR
9-Fluorenone-4-COOH	224	41.5	NR	40	100	NR
2-Methyl-1-tetralone	160	5.2	NR	NR	10	NR
2-t-Butyl phenol	150	6.3	NR	NR	10	10
2-Methyl-1,4-Naphthoquinone	172	9.8	NR	NR	10	NR

NR = not reported.

TABLE 13
Comparison of Categories in Test Mixture LC6 and Test Spectrum LC6b Identified by LRMS

		Category	Weight %	Contractors			
				A	B	C	D
Mixture	LC6	Ketone*	41.1	200	100	30	10
		Acid*	52.5	NR	40	110	NR
		Phenol	6.3	NR	NR	10	10
Spectrum	LC6b	Ketone*	22.6	NR	NR	NR	111
		Acid*	43.9	NR	†	110	NR
		Phenol	11.2	100	NR	NR	NR

*9-Fluorenone-4-carboxylic acid was placed in acid category.

†Compound reported but intensity not given.

NR = not reported.

TABLE 14
Low Resolution Mass Spectral Interpretation Inputs and How They Are Used By Contractors

	A	B	C	D
The analyst:				
1. Looks for molecular ions	✓	✓	✓	✓
2. Looks for some fragment ions	✓	✓	✓	✓
3. Looks for eight most abundant ions	✓	no	✓	no
4. Uses eight-peak indices often	✓	no	✓	no
5. Accounts for all peaks above given intensity and m/e value	✓	no	✓	no
6. Uses peak intensity ratios	✓	no	no	✓
7. Finds homologous series very important	✓	no	no	✓
8. Gives IR data equal weight	no	no	no	no
9. Uses LC data to verify & exclude	✓	✓	no	✓
10. Uses LC data to verify only	no	no	✓	no

✓ = agrees with input shown.
no = does not agree with input shown.

TABLE 15
Examples of How IR Data Were Used to Verify and/or Revise LRMS Results

Contractor	Sample	Before IR	After IR
A	LC5	anthrone	deleted anthrone
	LC6	hydroxy-anthraquinone	9-fluorenone-4-COOH, added fluorene-COOH
B	LC6a	nothing	pyridone
	LC5a	acridine fluorenone	carbazole ethyl carbazole
C	LC4a	anthracene	anthracene-major component
	LC5a	carbazole	carbazole confirmed
D	LC6	nothing	aromatic acids
	Tar LC6	only heterocyclic N compounds	heterocyclic N compounds, aromatic acids, esters, ketones

PB82-211624

CHARACTERIZATION OF COAL GASIFICATION BY-PRODUCTS AND AMBIENT AIR SAMPLES FROM A LURGI GASIFICATION FACILITY BY SELECTIVE DETECTOR GAS CHROMATOGRAPHY

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ABSTRACT

Samples of primary by-products produced by the Lurgi Gasification Plant in the Kosovo Region of Yugoslavia were taken for organic characterization. Ambient air around the plant was monitored for particulates and organic vapors.

Primary by-products, organic vapors, and particulate-borne organics were analyzed for sulfur- and nitrogen-containing organic compounds using selective-detector gas chromatography. Selective detection revealed a positive correlation between sulfur- and nitrogen-containing compounds in the primary by-products and the ambient air samples. Relative quantification and tentative identification of the major sulfur- and nitrogen-containing compounds was established by comparison to standards and by peak enrichment techniques. These identifications were confirmed by GC/MS.

INTRODUCTION

An ambient air monitoring program was conducted at the Kosovo industrial complex in Yugoslavia. This complex contained a Lurgi gasification plant that produces medium BTU gas from lignite. This site was selected because Lurgi technology is proposed for indirect liquefaction and SNG plants in the United States. The main problem within this program was to correlate the emissions from the gasification plant to the components found in the ambient air samples. The samples collected were particulates on HiVol quartz fiber filters and organic vapors on Tenax resin.

A phased analytical approach was developed to solve the correlation problem. The analytical techniques in this program were verified before analyses were begun. Extraction and thermal desorption efficiencies were determined and reproducibility verified. Vapor breakthrough on the Tenax resin traps was determined for various organic compounds.

Within this program the analytical procedures involving gas chromatography were used for screening of samples and preliminary identification of organic compounds. Retention times and spiking with known compounds were used for tentative identifications. A Hall detector in the nitrogen and sulfur specific modes was used to profile the components in both the gasifier by-products and air samples. These profiles were compared in order to provide the correlation between the gasification plant emission sources and the air samples.

SAMPLING

HiVol Filters

Ambient air samples for particulates were taken on HiVol quartz filters. The schematic diagram shown in Figure 1 illustrates where the filter is located in the sampling apparatus. Typical parameters for particulate sampling were:

Sampling rate $\cong 1m^3$/min.;

Sample volume $\cong 1400m^3$/24 hr. (25°C, 1 atm.).

The filters were weighed before and after sampling for particulate loading. However, for organic analysis, the filter and particulates were stored in organic-free aluminum foil for organic analysis.

Tenax Samples

The following is a description of the Tenax samples. Figure 1 illustrates the location of the Tenax traps in the sampling apparatus. The traps contained $\sim$3g of 60/80 mesh Tenax resin. The second trap was used as a back-up to indicate breakthrough of compounds from the first trap. Typical sampling parameters for the Tenax traps were:

Sampling rate $\cong$ 4ℓ/min.;

Sample volume $\cong 5.8m^3$/24 hr. (25°C, 1 atm.).

The Tenax traps after sampling were sealed with Teflon end caps in the field. When the samples arrived at the laboratory, the Tenax resin was transferred to a clean, 40 mℓ glass vial with a Teflon-lined cap. The weight of the resin was obtained during the transfer.

TREATMENT OF SAMPLES

A phased approach was used to select and analyze the ambient air samples. This approach is outlined as follows:

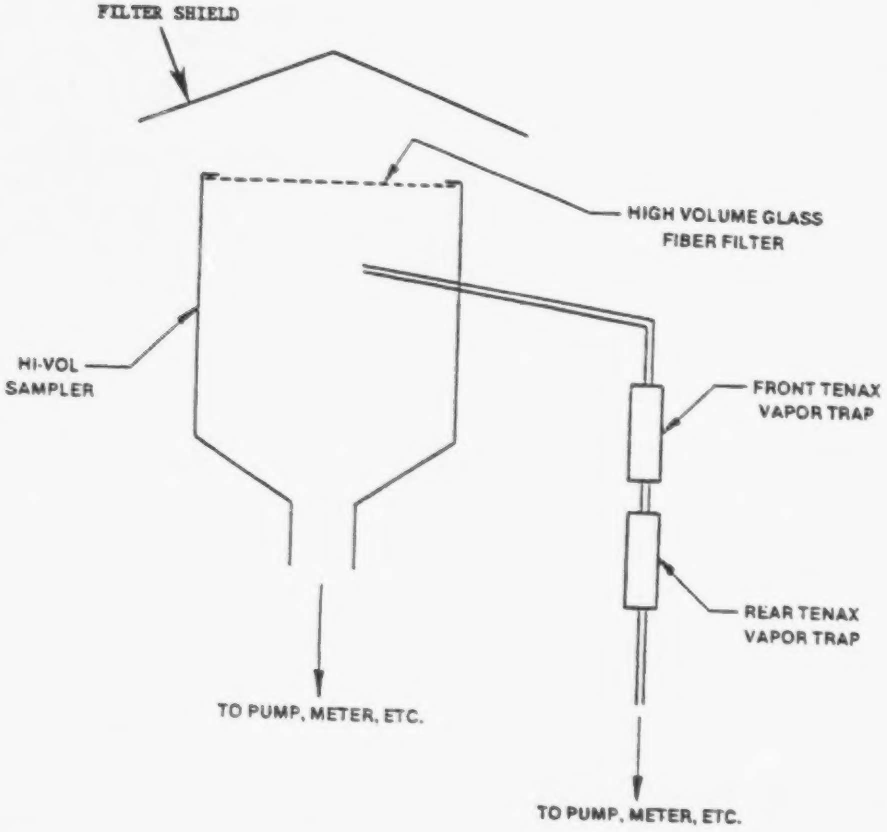
1) Secure and identify all samples;

2) Interpret meteorological data and select samples;

3) Selectively screen several upwind, downwind and blank samples for organics using a gas chromatograph (GC) equipped with a flame ionization detector (FID);

4) Using an FID and several selective detectors, obtain GC profiles of selected by-products from the gasification plant;

FIGURE 1 CONFIGURATION FOR COLLECTING AEROSOL SAMPLES FOR ORGANIC ANALYSIS


FILTER SHIELD
HIGH VOLUME GLASS FIBER FILTER
HI-VOL SAMPLER
FRONT TENAX VAPOR TRAP
REAR TENAX VAPOR TRAP
TO PUMP, METER, ETC.
TO PUMP, METER, ETC.

5) From screening data, carefully select samples to be profiled and quantified by gas chromatography using an FID and Hall detector (sulfur and nitrogen modes);

6) Compare profiles and establish correlations if possible;

7) Input all data with tentative identifications to the mass spectroscopist for identification, confirmation, and quantitation of major compounds.

In the final treatment of data, the concentrations of compounds in the air samples were plotted versus the percent downwind of that sample. Percent downwind was defined as the percent of the total sampling time in which the sampling site was within a 45° corridor of the downwind direction. From the plots, a correlation coefficient can be calculated.

An important aspect of this program was the verification of methods. The methods, verifications, and quality control are discussed in the following sections. After these sections, the application of these methods to the Lurgi gasification facility are outlined.

Extraction of Samples

Both Tenax and HiVol filter samples were Soxhlet extracted for 24 hours. A 1.0 to 1.5 gram portion of a Tenax sample was extracted. Cyclopentane was the solvent used with the Tenax samples, and dichloromethane was used for the HiVol filters. Since the dichloromethane produces noise in the Hall detector, the solvent was exchanged with cyclopentane before injection.

Thermal Desorption of the Tenax Samples

Thermal desorption was used as a second, independent method for separating the organic vapors collected on the Tenax resin, prior to the GC-FID profiling. Approximately 0.05g aliquots of the homogenized Tenax resin were placed in a thermal desorption injector mounted on a GC-FID. The adsorbed vapors were thermally desorbed from the Tenax and collected in a pre-column trap held at dry ice/acetone temperature. At the completion of desorption, the coolant was removed from the cold trap and the GC oven program was initiated.

Gas Chromatographic Conditions

Table 1 contains a list of conditions and materials used for the various detectors on the gas chromatographs. Note that similar chromatographic conditions have been used so that chromatographic profiles can be compared.

Two packed columns were necessary for this work. For most hydrocarbons and sulfur-containing compounds the OV101 phase worked well. The polar nitrogen-containing species tailed on this phase; therefore, a 10% Apiezon L plus 2% KOH mixed phase was used.

TABLE 1

Conditions Used with the Three Different Detectors (Modes)

Detector (Mode):	Flame Ionization Detector (FID)	Hall-Detector Sulfur (HECD-S)	Hall Detector Nitrogen (HECD-N)
Condition			
Carrier Flow	N_2, 30 ml/min.	He, 35 ml/min.	He, 35 ml/min.
Injector Temperature	250°C	250°C	250°C
Detector Temperature (Reactor)	300°C	225°C (850°C)	225°C (850°C)
Oven Program	50°C for 5 min; 20°/min to 250°C, hold for 60 min.	50°C for 5 min; to 250°C, hold for 60 min.	50°C for 5 min; to 250°C, hold for 60 min.
Column	10% OV101 on 80/100 mesh Supelcoport; 10'x2 mm ID glass column	10% OV101 on 80/100 mesh Supelcoport; 10'x2 mm ID glass column	10% Apieson L, 2% KOH on 80/100 mesh Chromosorb, W-AW, 10'x2 mm ID glass column

Tentative Identification

Two techniques were used to assign tentative identifications to organic compounds in the samples. The first involved spiking known compounds into samples for peak enrichment and identification. This technique was successful in the tentative identification of benzene, toluene, xylenes, several of the multiple ring aromatics and four of the alkyl substituted thiophenes and benzothiophene. The second technique was a simple plot of boiling point versus retention time of normal alkanes. Using this technique to predict the boiling point of unknown compounds coupled with the knowledge of what to expect as emissions from the gasifier, tentative identifications were made. This technique was especially successful with non-polar compounds.

METHODS VERIFICATION

Both thermal desorption and Soxhlet extraction were tested with model compounds spiked onto both the fiber filters and the Tenax resin. The spiked samples provided data on reproducibility and efficiency of recovery. Ambient air Tenax samples provided data on breakthrough during sampling as well as reproducibility of the thermal desorption technique.

Recoveries for Solvent Extraction and Thermal Desorption

Blank glass fiber filters and blank Tenax resin aliquots were spiked with five aromatic compounds before solvent extraction: toluene, naphthalene, anthracene, 1,2-benzanthracene and benzo(a)pyrene. The recoveries of these compounds were measured by GC-FID analysis of the extracts and the spiking solution. All measured recoveries fell in the range of 80-120%, except for toluene. The toluene recovery was generally in the range of 20-70%, and was probably due to losses occurring in the solvent evaporation step.

Blank Tenax resin was spiked with benzene, toluene, naphthalene, anthracene and 1,2-benzanthracene prior to thermal desorption. The recoveries for these compounds by thermal desorption and GC-FID were in the range of 90-110%.

Vapor Breakthrough on the Tenax During Sampling

The front and rear Tenax traps were screened for sample vapor breakthrough by GC-FID. Equal quantities of benzene and tolune were found on the front and rear traps indicating complete vapor breakthrough for these two species under the conditions used in this sampling. For the C_2-alkyl-benzenes, the quantity on the rear trap was only about 15% of that on the front trap and for heavier species, the quantity on the rear trap was negligible. Thus, breakthrough was a problem for benzene and toluene and compounds of similar or higher volatility.

Reproducibility of the Thermal Desorption Technique

The precision of the thermal desorption method was determined by quantifying thirteen corresponding peaks in a duplicate measurement by GC-FID of weighed Tenax sample aliquots. The peaks selected included benzene, toluene, the C_2-alkyl benzenes and naphthalene plus eight other prominent peaks not identified at the time. The reproducibility in this duplicate determination was good, with an average percent standard deviation for all thirteen components of 8.7%.

APPLICATION

The phased scheme was applied to an ambient air sampling program involving a Lurgi coal gasification facility. The Hall detector scans in both the sulfur and nitrogen modes will be discussed as specific examples of results from this type of approach. The details of the methods and a complete discussion of the results will appear in the final contract report to the Environmental Protection Agency.

Selection of Samples

The phased approach outlined in the Treatment of Samples section was followed. After identifying all the samples, they were categorized as to upwind, downwind and crosswind samples from the meteorological data. For screening purposes, two downwind Tenax samples with the highest percent downwind during the sampling period were chosen. These were coupled with their corresponding upwind samples, one low downwind sample (crosswind) and two blanks. The flame ionization detector (FID) scans of these samples confirmed the choices made as far as organic loading was concerned.

Analyses

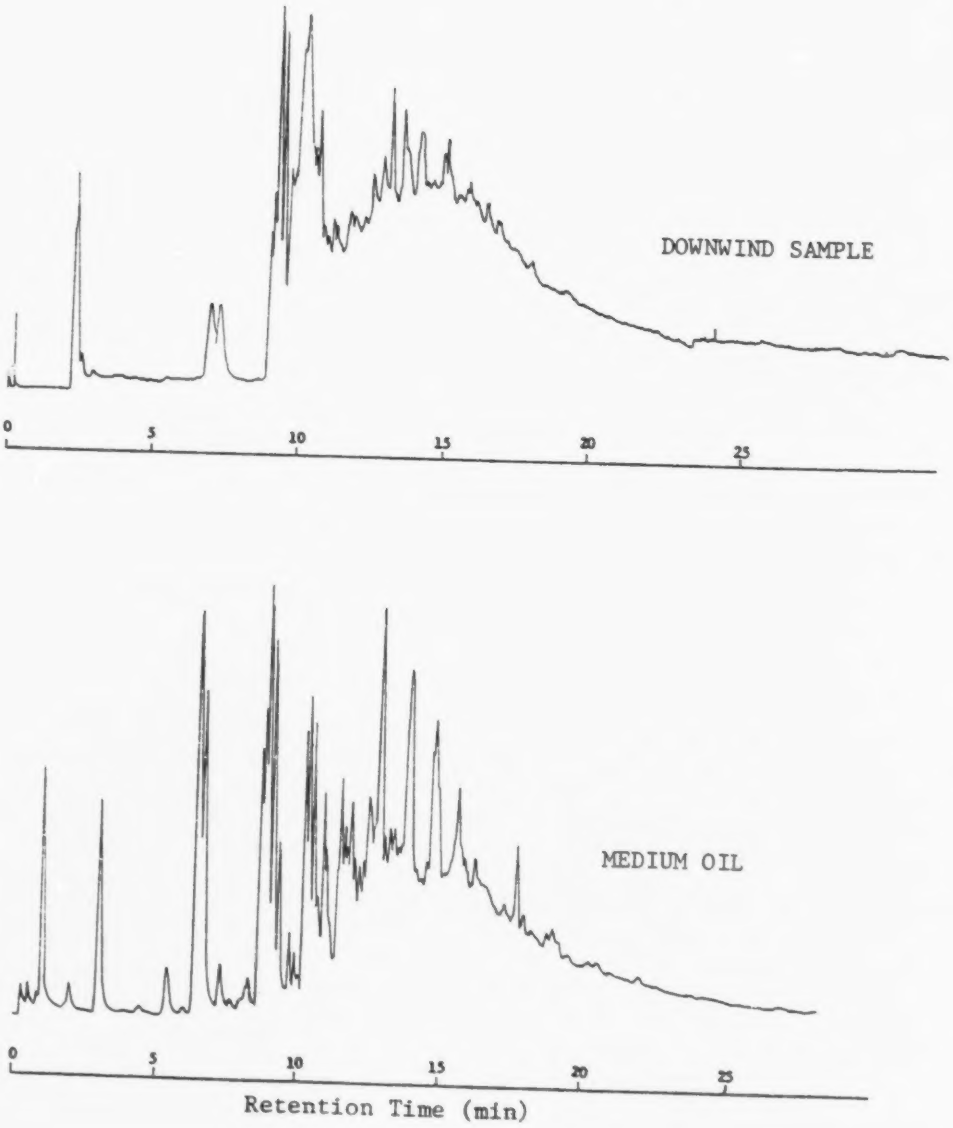
After screening, approximately 1g of each of the selected Tenax samples was Soxhlet extracted for 24 hours with cyclopentane. The solvent was reduced in volume to 0.5 ml in a Kuderna-Danish apparatus using a 3-ball Synder column. The samples along with standards and by-products were profiled on gas chromatographs equipped with FID, Hall-sulfur and Hall-nitrogen detectors. The conditions for these analyses were discussed in the Treatment of Sample section.

Results: Sulfur Species

Two representative profiles of the sulfur-containing species are illustrated in Figure 2. The chromatograms show the distribution of sulfur species in the "medium oil" by-products from the gasification plant and a typical downwind air sample. The retention times are slightly different for the compounds due to variations in the initial temperature of the gas chromatograph, but the profiles have a high degree of correlation.

The two large peaks at about 6.5 minutes in the "medium oil" were tentatively identified by spiking. The compounds, 2- and 3-methylthiophene, were spiked into the sample and the peaks which increased relative to

FIGURE 2 GC-HECD SULFUR COMPOUND PROFILES FOR A DOWNWIND TENAX VAPOR TRAP EXTRACT AND FOR MEDIUM OIL



the others were identified as the spiked compounds. This technique was also used for thiophene (RT ∿ 3 min.), 2,4-dimethylthiophene (RT ∿ 9 min.), ethylthiophene (RT ∿ 9 min.), and benzothiophene (RT ∿ 13 min.). Thiophene was not detected in the Tenax sample, perhaps because of its high volatility and subsequent low collection efficiency on Tenax resin or loss in solvent evaporation.

The resin and/or solvent contained an interference which masked part of the profile around 10 minutes. The upwind site Tenax sample and the blank sulfur profiles are given in Figure 3 and are essentially identical. That is, no sulfur species were detected in the upwind vapor sample corresponding to the downwind sample. We concluded that the profiles of sulfur species exhibit a positive correlation between the downwind air samples and the gasification plant.

Results: Nitrogen Species

Very similar results were found with the nitrogen components in the "medium oil" by-product and the air samples. In Figure 4, the profiles of "medium oil" and a downwind sample are illustrated. The profiles are similar; however, the large, early peak in "medium oil" at 6.7 minutes is not present in the air sample. Again, this may indicate that the more volatile compounds had low collection efficiencies on the Tenax or they were lost during concentration of the solvent.

Most of the nitrogen compounds in the "medium oil" and the air samples appeared to be alkyl substituted pyridine(s) or quinoline(s) from spiking and boiling point identifications. One of the peaks at about 15.7 minutes retention time and another at about 18.5 minutes were also seen in the upwind samples but not in the blank resin (Figure 5). The peaks were either low in concentration in the "medium oil" or not in the "medium oil" at all. This indicated that the source of these compounds was not the gasification plant.

Results: GC/MS

Most compounds tentatively identified by GC were confirmed by GC/MS. However, nitrogen compounds in the "medium oil" were not confirmed initially because of the carbon 13 abundance in carbon-containing analogs. An acidic, aqueous extraction of "medium oil" and a neutral back-extraction separated the carbon-containing analogs from the nitrogen-containing compounds.

Scans with an HECD-N of the basic fraction from "medium oil" gave an identical chromatogram to direct injection. Therefore, no major nitrogen-containing compounds were lost in the extraction process. This also indicated that all the major chromatographable compounds which contain nitrogen are basic. GC/MS was able to confirm all tentatively identified peaks in the "medium oil" using the basic fraction.

FIGURE 3 GC-HECD SULFUR COMPOUND PROFILES FOR AN UPWIND AND A BLANK TENAX VAPOR TRAP EXTRACT

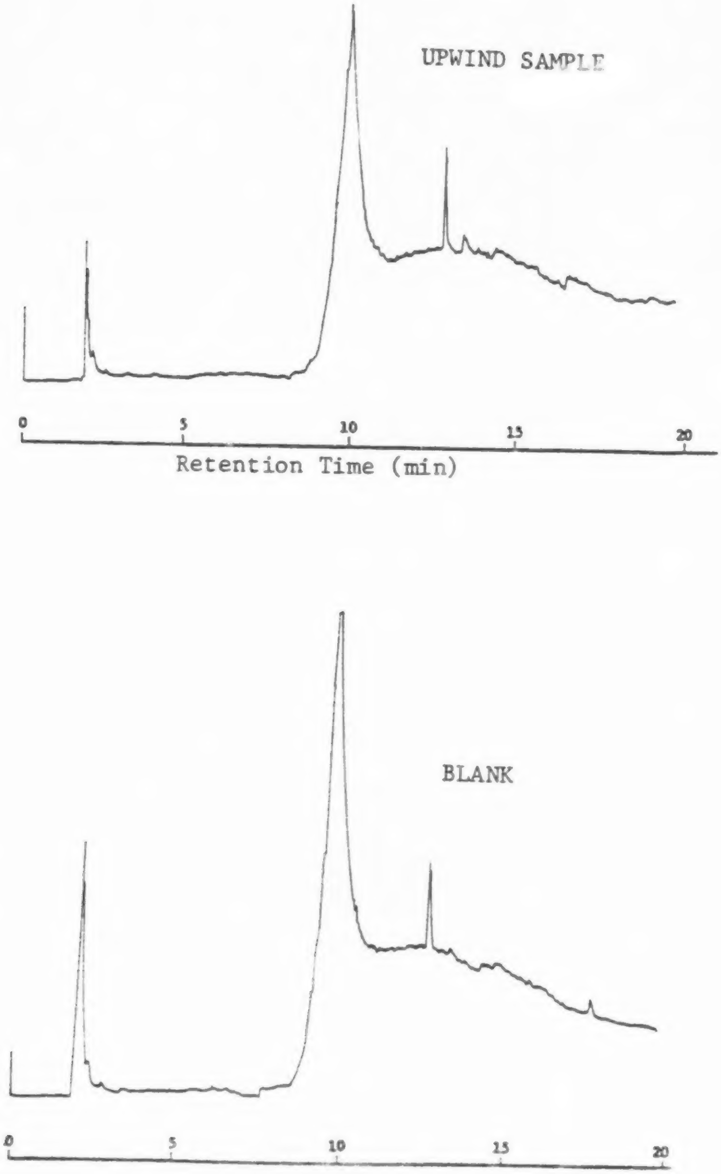


FIGURE 4 GC-HECD NITROGEN COMPOUND PROFILES FOR A DOWNWIND TENAX VAPOR TRAP EXTRACT AND FOR MEDIUM OIL

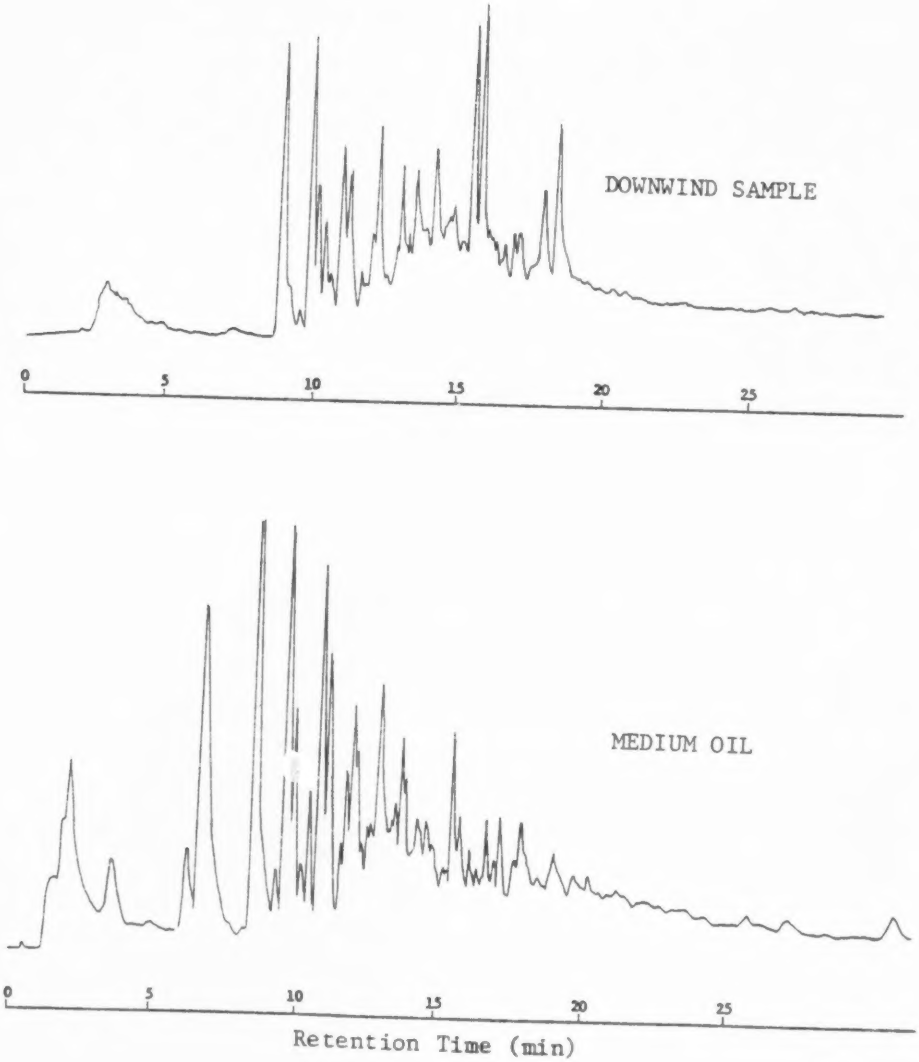
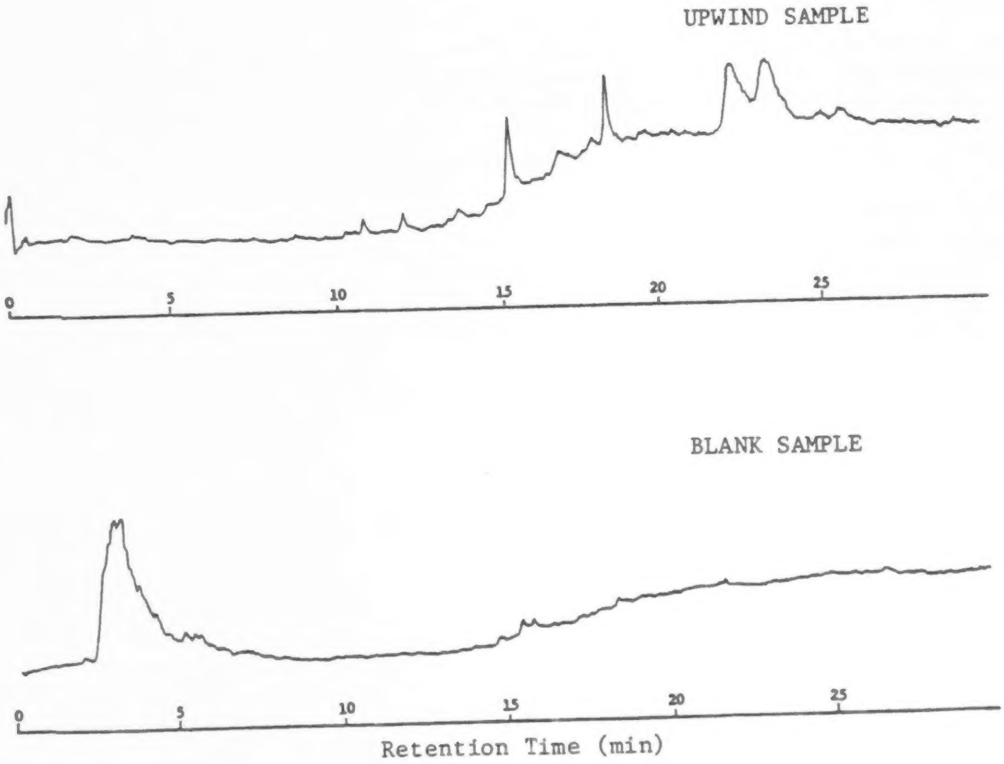


FIGURE 5 GC-HECD NITROGEN COMPOUND PROFILES FOR AN UPWIND AND A BLANK TENAX VAPOR TRAP EXTRACT



CONCLUSIONS

The following conclusions were obtained from the application of the phased analytical approach:

1) Positive correlations between the ambient air samples and the gasification plant products were established from the nitrogen and sulfur profiles.

2) The phased approach was effective in characterizing samples containing a wide range of organic compounds including hydrocarbons, sulfur compounds and nitrogen compounds.

The phased approach should be applicable to other processes where selective detection can be applied. By using selective detectors, GC/MS time can be more effectively used with a decrease in analytical costs.

ACKNOWLEDGMENTS

This program was funded by the United States Environmental Protection Agency under contract numbers 68-02-2608 (Task 42) and 68-02-3137. Ronald K. Paterson (EPA) was most helpful during all phases of this work. Without the support of T. Kelly Janes (EPA) and William J. Rhodes (EPA), the analyses of the gasification by-products would not have been possible.

PB82-211632

CHARACTERIZATION OF PROCESS STREAMS FROM LIQUEFACTION OF LOW-RANK COAL WITH SYNTHESIS GAS

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ABSTRACT

Beulah (ND) seam lignite was liquefied with 1:1 carbon monoxide-hydrogen at 460° C and 27.5 MPa in a 2.3 kg coal/hour continuous flow reactor. Process streams obtained included C_1 to C_4 hydrocarbon gases, water effluent, light oils, vacuum distillate, THF soluble vacuum bottoms, and THF insoluble fraction. Analysis of tail gases was by on-line GC. Light oils (volatile at 27.5 MPa and 300° C) were separated into phenolic, basic, hydroaromatic and hydrocarbon fractions by solvent extraction. These were analyzed by 200 MHz ^1H and 50 MHz ^{13}C NMR, GC-MS, low voltage MS, IR, UV, fourth derivative UV, and capillary GC. Water inherent in the lignite formed a separate layer in the light oil collector. The water was characterized for pH, NH_3, alkalinity, cyanide, sulfide, TOC, phenol and cresol content. Prior to vacuum distillation the bulk product was sampled and analyzed by NMR, % water, % ash, % CHNS, oxygen by neutron activation, THF solubility, viscosity, molecular weight distribution of the THF solubles by gel permeation HPLC, and ash analysis by x-ray fluorescence. Major inorganic components were determined by inductive coupled argon plasma spectrometry and atomic absorption after wet ashing. Vacuum distillate was characterized by column chromatography on alumina, solvent extraction, HPLC, NMR, IR, UV and LVMS. Solids deposited on the reactor walls (coke) were examined by optical microscopy, scanning and analyzing electron microscopy, and x-ray fluorescence.

INTRODUCTION

Pioneering work on the liquefaction of a finely ground slurry of lignite utilizing synthesis gas (1:1 carbon monoxide-hydrogen) was carried out by Appell (1) and co-workers at the Pittsburgh Energy Technology Center using a rocking bomb autoclave. A continuous process liquefaction unit was also operated at Pittsburgh (2). In 1975 the study of liquefaction of low-rank coal was transferred to the Grand Forks Energy Technology Center (GFETC). A hot charged time sampled stirred autoclave system was developed and used to study the reaction kinetics of the process (3). A 2.3 kg coal per hour continuous process liquefaction unit was then constructed at GFETC, and is presently being used in the study of liquefaction of low-rank coal by adaptations of various current processes (4, 5).

Extensive deposits of lignite are found in the Northern Great Plains Province (including western North Dakota, eastern Montana and southern Saskatchewan) and the Gulf Coast Province (a narrow belt across Texas, Louisiana and Arkansas). The lignites differ from higher rank coals by containing high moisture, low sulfur and alkaline inorganic components.

Much of the inorganic matter consists of alkali and alkaline earth cations bound as humate salts at ion exchangeable sites on the coal structure. Minerals such as pyrite, clay and silica are finely dispersed throughout the organic matrix. Lignites also have a much higher organic oxygen content than higher rank coals. The unique properties of lignite are believed to affect its liquefaction behavior so that processes developed for bituminous coal are not directly applicable to lignite without further research.

REACTION CONDITIONS

Current emphasis in coal liquefaction is toward formation of distillable products. Experiments with the continuous reactor at GFETC have included recycling of the heavy ends including their mineral content, and removal of the volatile oils as products of each pass. A 4.55 liter stirred autoclave reactor was operated at 460°C and 27.5 MPa . The pasting solvent for the first pass was redistilled anthracene oil (IBP 296° C @ 1.3 KPa), a phenol-free distillate by-product of bituminous coke production. Slurry was prepared for each pass through the reactor by adding 30% by weight, pulverized (100% minus 60 mesh) as-received (ca. 30% moisture) lignite to the heavy product (BP 300°C @ 27.5 MPa) from the previous pass including the mineral matter and any unreacted lignite. The feed coal was a Beulah, ND seam lignite with unusually high mineral content. Table 1 contains the analyses of the feed coal. Slurry was fed to a 4.55 liter autoclave acting as a continuous stirred tank reactor (CSTR) to obtain a nominal residence time of one hour. CO and H_2 gas (1:1) were added at 14.2 l/min. Further details of the reactor conditions have been presented by Farnum et. al. (6).

The yields of various process streams produced during product slurry recycle operation are illustrated in Figure 1 as a function of pass number. The overall yields during 34 passes were: C_1-C_4 hydrocarbons, 19%; high pressure volatile oils, 19%; vacuum distillate, 36%; THF soluble vacuum bottoms, 9%; and THF insoluble organic polymers, 11%. These were calculated on a moisture- and mineral matter-free coal basis. The process was upset during pass 16 by plugging of the reactor with "coke" consisting of mineral particles dispersed in an organic polymer matrix, causing the yield of organic THF insolubles exiting the unit to drop to zero during the pass. The unit was restarted using the heavy oils product as pasting solvent, and operation was continued through 34 passes. Figure 2 illustrates the approach to constant composition of the light oils in terms of aromatic-to-aliphatic proton ratio measured both by proton NMR and infrared absorbance. Samples from the thirtieth pass were selected for detailed chemical characterization studies as representative of lignite-derived products.

WATER EFFLUENT

Lignite seams often serve as aquafiers and contain 35 to 40% water when freshly mined. After handling and pulverizing, the feed coal in this experiment was 29% water by weight. During liquefaction the moisture makes an undesirable contribution to total pressure within the reactor, but is available for conversion of CO to hydrogen via the water gas shift reaction. Thirty-one percent of the water in the feed slurry was recovered as a layer which separated from the light oils which condensed from the gas phase in the product receiver. This effluent water was characterized by standard analytical methods for waste water and standard EPA methods (7, 8). The average of analyses of four samples of condensate water produced under lined-out conditions is reported in Table 2.

LIGHT OILS

The light oils which were condensed from the product in the gas phase at 300°C and 27.5 MPa were fractionated by solvent extraction into four chemical classes using the method of Fruchter et al. (9) modified by the use of pentane rather than isooctane. The use of methylene chloride following pentane extraction was also explored and found to give simpler mixtures in the case of the phenolic fraction. Characterization of the light oils by Farnum et al. is discussed in a forthcoming preprint (10). The fractionation scheme is illustrated in Figure 3.

The light oil fractions were separated by capillary GC using a 50 meter OV-101 glass column. The number of components detected is listed in Table 3. There are less than 85 components above 1%, allowing for some overlap of components among fractions.

The ultraviolet spectrum of the light oil from the thirtieth pass (labeled LO 28-14) is displayed in Figure 4. Subtraction of the spectrum of the hydroaromatic fraction (HA) yielded a spectrum displaying many of the features of that of the phenolic fraction. The fourth derivative UV spectra displayed in Figure 5 show a maximum at 275 nm which is assigned to phenol, and a maximum at 268 nm assigned to the cresols. The maximum at 250 nm in the HA fraction is assigned to phenanthrene. Fourth derivative UV is useful in the analysis of complex mixtures since the maxima in the fourth derivative correspond to the maxima in the underivatized UV. There is a small shift in wavelength during derivatization, in this case about 4 nm towards shorter wavelength.

The investigation of the light oil fractions by ^{13}C NMR and ^{1}H NMR along with gas chromatography alone gave considerable information.

The two phenolic fractions, >20%, were well characterized. The CH_2Cl_2 fraction contained only the four most water soluble phenols, phenol itself, o-cresol, m-cresol and p-cresol, with a small amount of an unidentified phenol. The IR was consistent with this observation. The exact amounts present were determined by GC. The pentane soluble phenolic fraction also contained the same four phenols in large amounts with other alkylphenols making up the rest of the material (Figure 7a). There were no guiacols and no naphthols found.

The hydrocarbon fraction, 48%, is seen to exhibit ^{13}C resonance lines in the aliphatic region that coincide with the shifts seen for the normal paraffin series (Figure 6b). These may be long chain substituents on benzene or may be paraffinic hydrocarbons. Tetralin and decalin may be present. Methyl benzenes and methyl naphthalenes do not appear in this fraction.

The hydroaromatic fraction, 22%, Figure 6a and Figure 7b, shows prominent naphthalene lines in both the ^{1}H and ^{13}C NMR spectra. Phenanthrene is present as indicated by the low-field 4, 5 protons (Figure 7b). The IR shows the probable presence of ethers; however, the ethers are not aliphatic--i.e., methyoxy--in nature. The ^{13}C NMR does show the possible presence of dibenzofuran in very small amount. Carbazoles may be present.

The base fraction, 5%, contained phenolic contaminants since it was extracted first from an aqueous layer. It did concentrate most of the nitrogen compounds except for carbazoles. The quinolines and pyridines present in this fraction appear to have mainly methyl substituents. Two types of N-H stretching are seen in the IR, 3400cm^{-1} and 3485cm^{-1}. Protonated pyridine is seen at about 3400cm^{-1}.

HEAVY OIL

The product oil (less light oils) was characterized by gel permeation HPLC to ascertain the degree of molecular size reduction as a measure of liquefaction process efficiency. Figure 8 illustrates the approach to constant molecular weight distribution as the product oil is recycled with added coal. The use of the ratio of UV absorbance at 950 MW vs 250 MW as a liquefaction product parameter is illustrated in Figure 9, and has been previously discussed by Knudson et al. (11). Examination of the character and content of the vacuum bottoms from coal liquefaction has been discussed by Farnum et al. (12, 13).

Proton NMR in d$_5$- pyridine has been used to determine the aromatic (% Har), benzylic (% Hα) and other aliphatic (% Ho) proton distribution in the undistilled product slurry according to the method of Brown and Ladner (14). Table 5 lists the results along with some computed parameters, f$_a$ (the fraction aromatic carbon), σ (the degree of substition of aromatic sites) and Haru/Car (the H/C ratio of the hypothetical unsubstituted aromatic material). The approach to constant composition is apparent. Mass spectral analysis of vacuum distillates from lignite liquefaction have been discussed by Schiller (15). Further work is in progress on characterization of light oils and vacuum distillate by LVMS and GC-MS.

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TABLE 1

Analyses of Coal for
Beulah 3, North Dakota, Lignite

Basis of reported analysis:	Coal Analysis (GF-79-2147)		
	As-received	Moisture-free	Moisture- and ash-free
Proximate analysis, pct.:			
Moisture	29.48	--	--
Volatile matter	30.21	42.84	50.53
Fixed carbon	29.58	41.94	49.47
Ash	10.73	15.22	--
TOTAL	100.00	100.00	100.00
Ultimate analysis, pct.:			
Hydrogen	6.20	4.15	4.89
Carbon	42.87	60.79	71.71
Nitrogen	0.48	0.68	0.80
Oxygen	37.91	16.59	19.57
Sulfur	1.81	2.57	3.03
Ash	10.73	15.22	--
TOTAL	100.00	100.00	100.00

* An "atypical" Beulah Lignite chosen specifically for its high ash content.

TABLE 2

Effluent Water Composition[a]
(Averages of Recycle Passes 21, 24, 29 and 32)

	(Concentrations in ppm)
pH at analysis	8.6
Alkalinity as $CaCO_3$	80,400
Ammonia	27,300
Total sulfur	2,380
Total carbon	31,200
Inorganic carbon	12,400
Organic carbon	18,800
Phenol	6,430
o-cresol	579
m,p-cresols	1,640

a. Analysis carried out under contract by Stearns-Roger, Inc.

TABLE 3

Capillary G.C. Analysis of LO 28-14 Fractions

Fraction	# Components			
	>1%	0.5-1.0%	0.25-0.5%	Total
Basic	12	7	21	48
Phenolic	26	23	10	84
Heteroaromatic	21	18	19	124
Hydrocarbon	26	14	20	121

TABLE 4

Elemental Analysis Distribution of Light Oil 28-14 and its Fractions Based on LO as 100%

	% C	% H	% O	% N	% S	% H_2O
28-LO-14	84.34	10.69	0.37	3.70	0.22	0.23
Basic	19.94	2.64	0.09	3.35	0.01	--
Phenolic	19.77	2.49	0.25	0.03	0.01	--
Heteroaromatic	22.38	2.21	0.035	0.32	0.20	--
Hydrocarbon	22.26	3.35	0.001	0.00	0.01	--

TABLE 5

NMR Analysis of Unfiltered Product[a]

Sample	Pass No.	%Har	%Hα	%Ho	fa	σ	Haru/Car
27-Y1	1	33.4	28.6	38.0	0.65	0.30	0.76
27-B2B	3	35.8	28.5	35.7	0.67	0.29	0.77
27-B2D	5	38.0	28.6	33.5	0.69	0.30	0.76
28-2B	18	42.6	28.7	28.7	0.73	0.30	0.75
28-4B	20	43.4	29.3	27.3	0.74	0.28	0.74
28-6B	22	42.7	28.8	28.4	0.73	0.29	0.73
8B	24	43.1	28.3	28.6	0.74	0.29	0.74
10B	26	43.5	27.1	29.5	0.74	0.27	0.72
12B	28	43.0	28.6	28.4	0.74	0.28	0.72
13B	29	43.9	27.7	28.5	0.75	0.28	0.71
14B	30	43.1	28.3	28.6	0.75	0.29	0.70
15B	31	42.5	28.0	29.5	0.74	0.29	0.71
16B	32	44.1	28.2	27.7	0.75	0.29	0.70

a. Analyses performed on a 90 MHz Spectrometer located at the University of North Dakota Department of Chemistry.

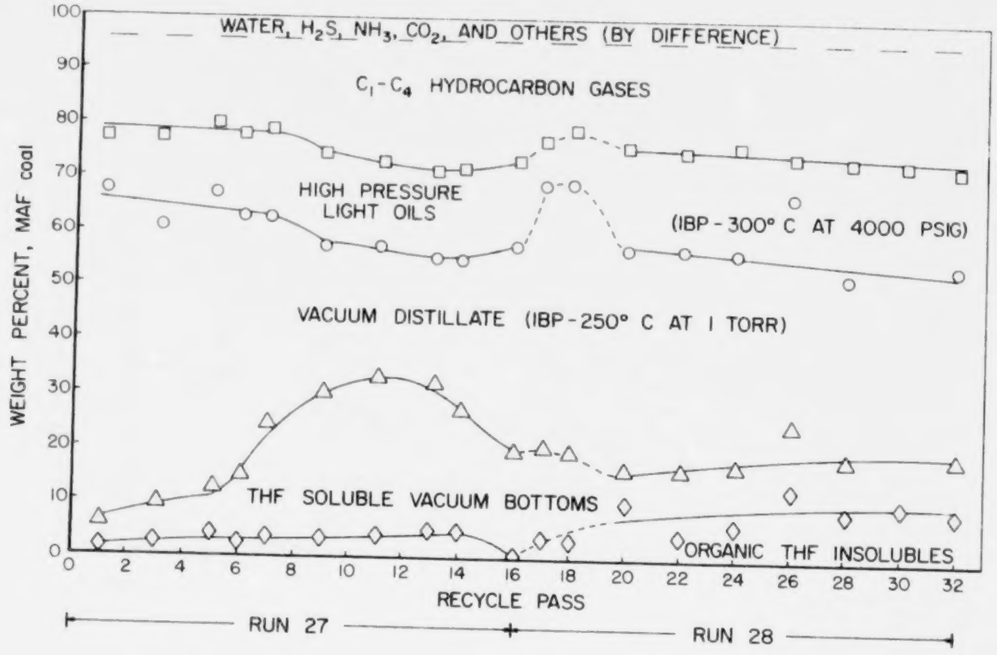

FIGURE 1 Runs 27 & 28: Distribution of MAF coal conversion products versus recycle pass.

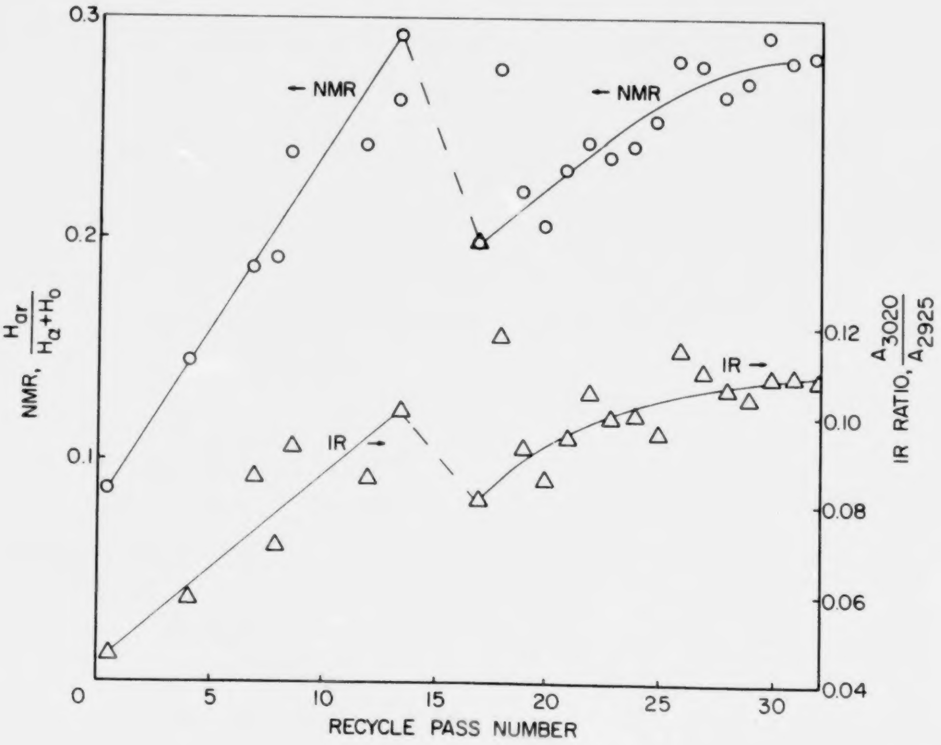

FIGURE 2 Aromatic to aliphatic proton ratio in light oils by NMR and IR versus recycle pass number.

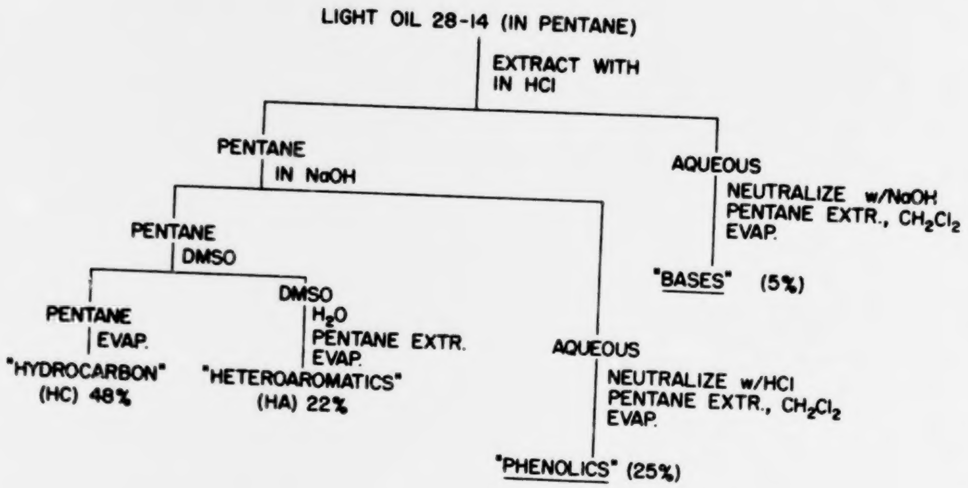
LIGHT OIL 28-14 (IN PENTANE)
EXTRACT WITH IN HCl
PENTANE
IN NaOH
AQUEOUS
NEUTRALIZE w/NaOH
PENTANE EXTR., CH2Cl2
EVAP.
"BASES" (5%)
PENTANE
DMSO
PENTANE
EVAP.
"HYDROCARBON" (HC) 48%
DMSO
H2O
PENTANE EXTR.
EVAP.
"HETEROAROMATICS" (HA) 22%
AQUEOUS
NEUTRALIZE w/HCl
PENTANE EXTR., CH2Cl2
EVAP.
"PHENOLICS" (25%)

FIGURE 3 Separation and recovery of the light oil fraction by extraction.

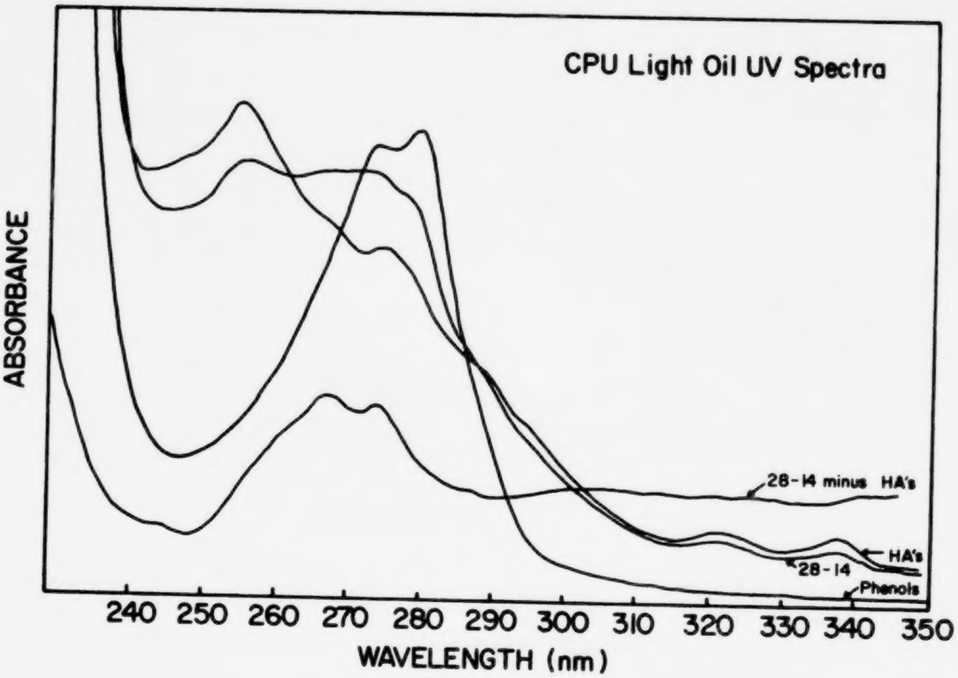

FIGURE 4 UV spectra of LO 28-14 and fractions.

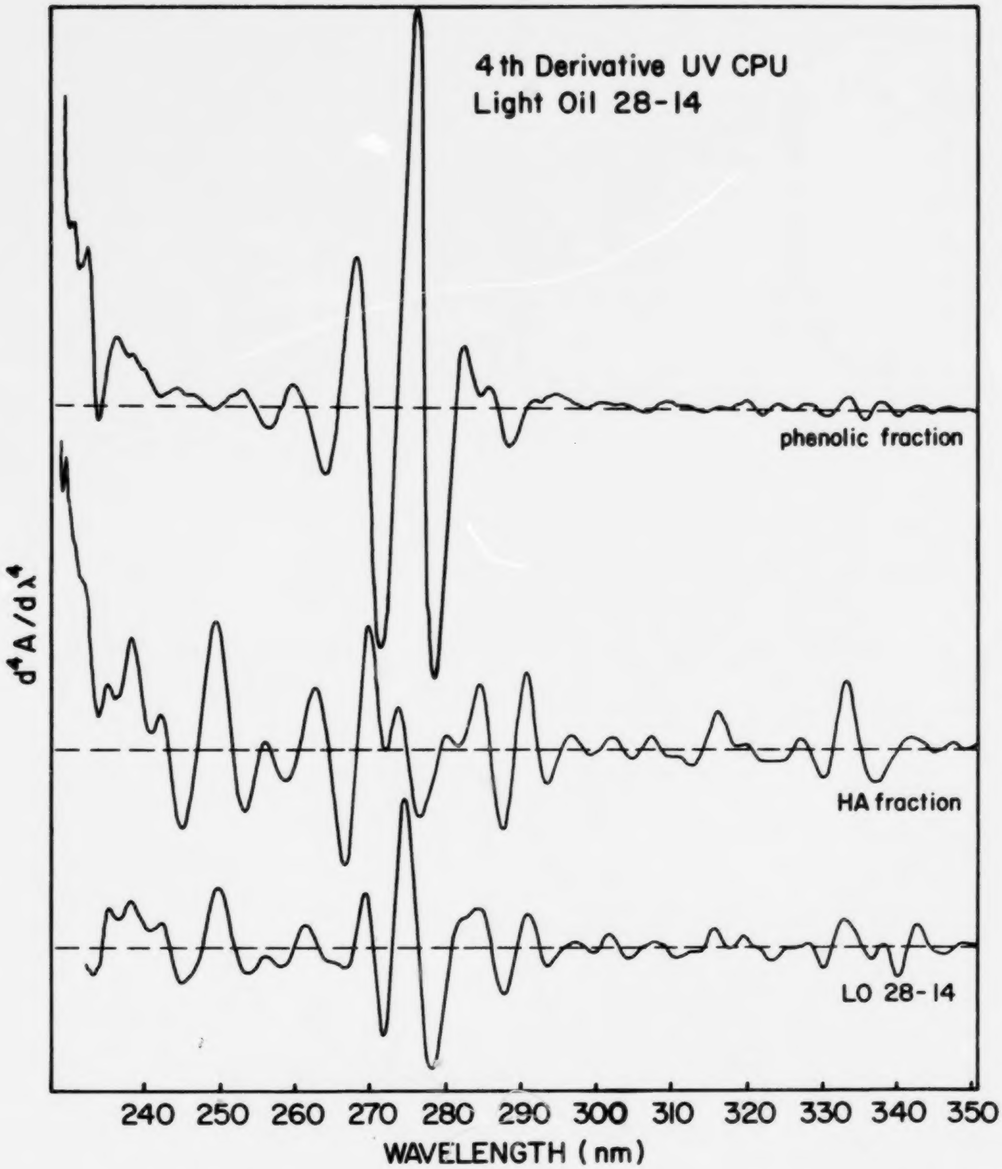

FIGURE 5 Fourth derivative UV of light oil 28-14 and fractions.

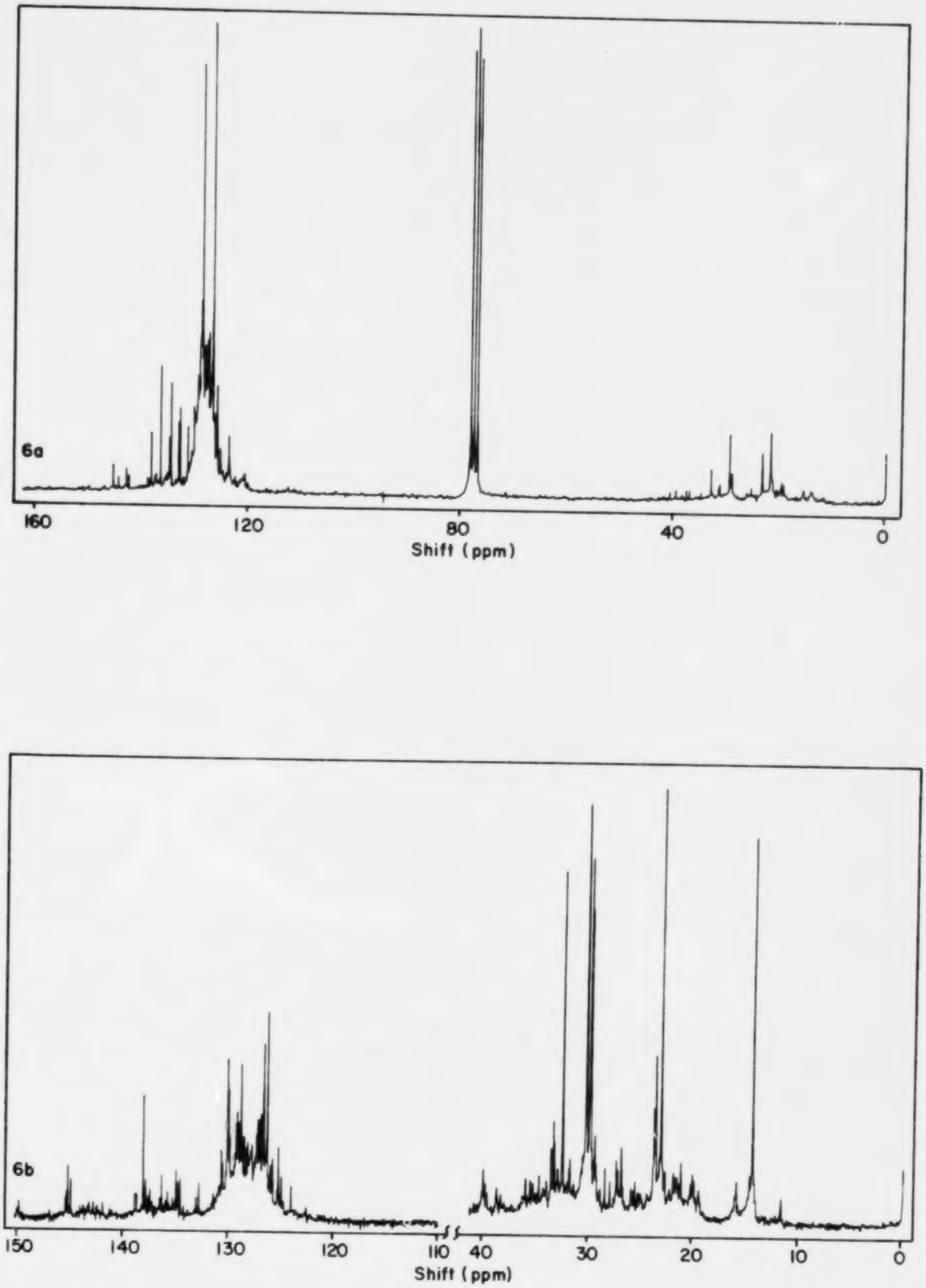

FIGURE 6 50 Mz ^{13}C NMR, a) of the hydroaromatic fraction, completely proton decoupled in CDCl$_3$, b) aliphatic and aromatic regions of the hydrocarbon fraction (without NOE, Cr(AcAc)$_3$, CDCl$_3$), of light oil 28-14.

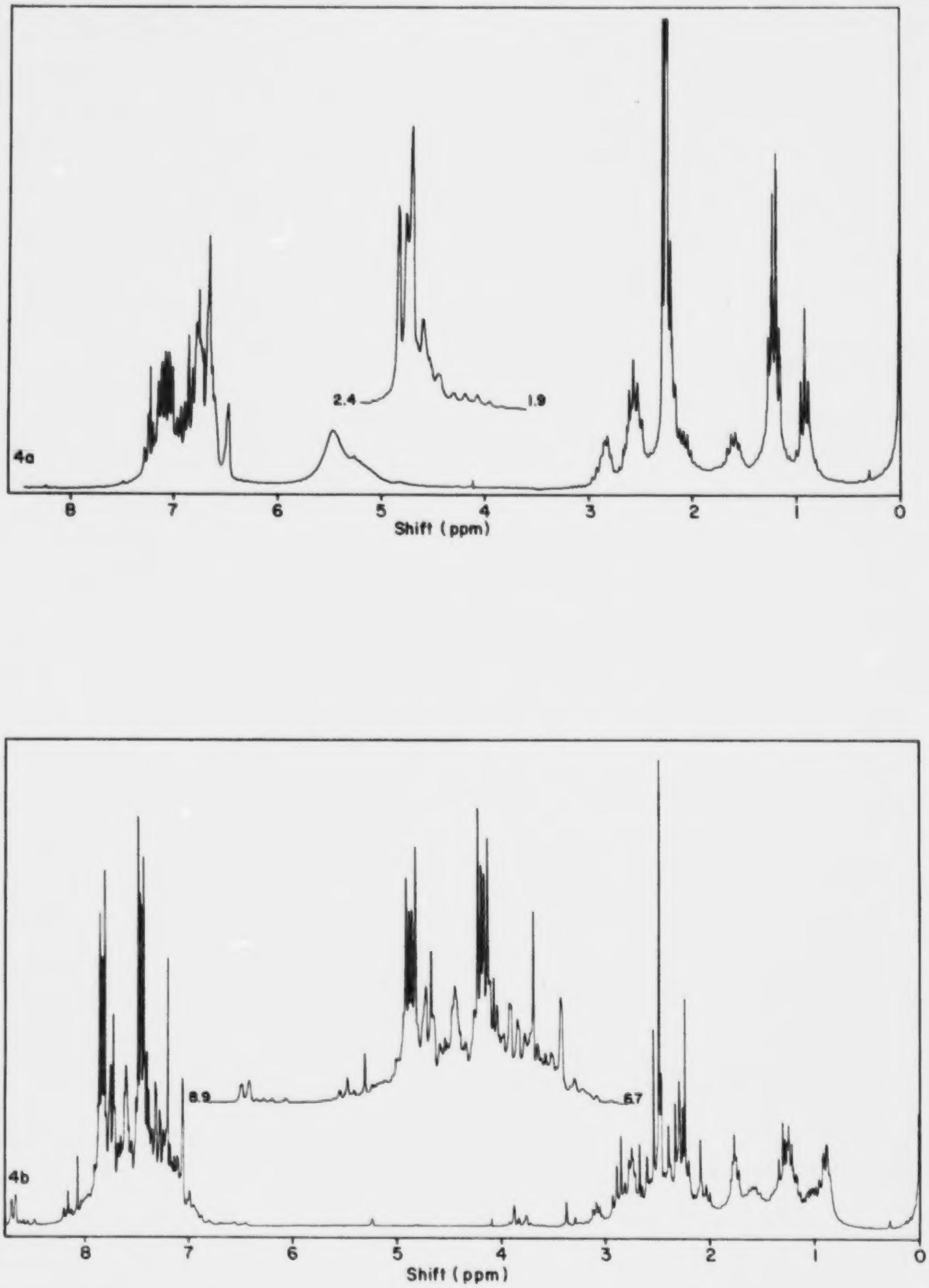

FIGURE 7 200 MHz ^1H NMR of light oil 28-14, a) phenolic fraction, b) hydroaromatic fraction.

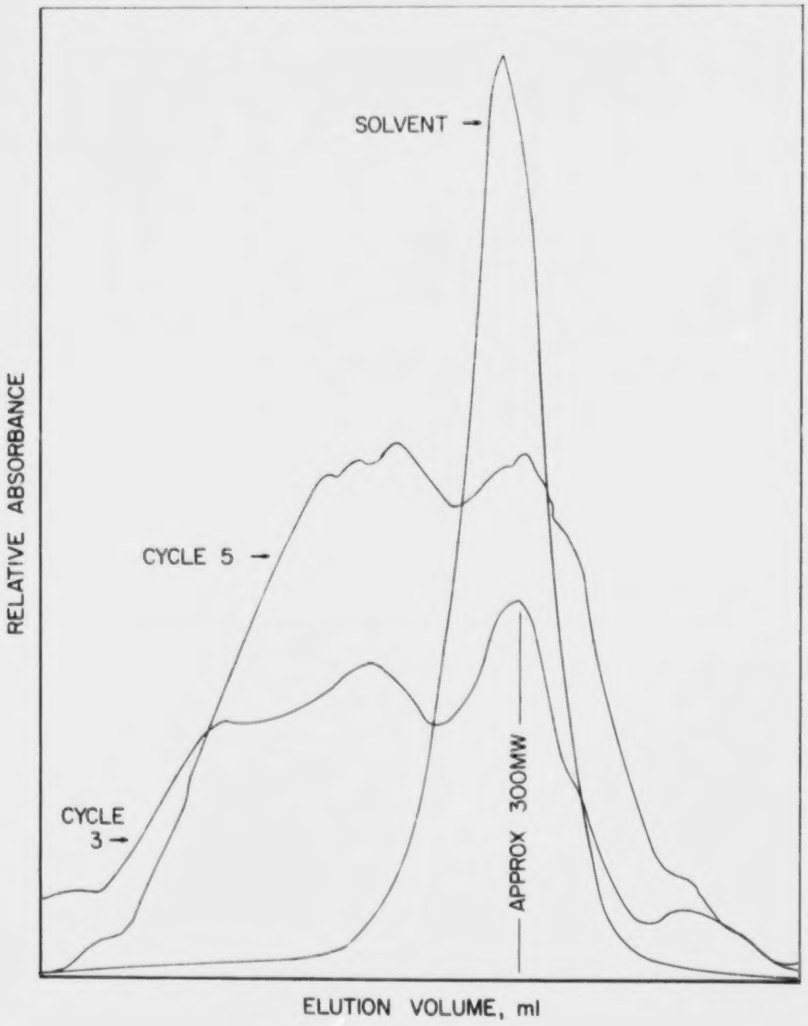

FIGURE 8 Changes in the molecular weight distribution with recycle (Run 27). Detection was at 365 nm.

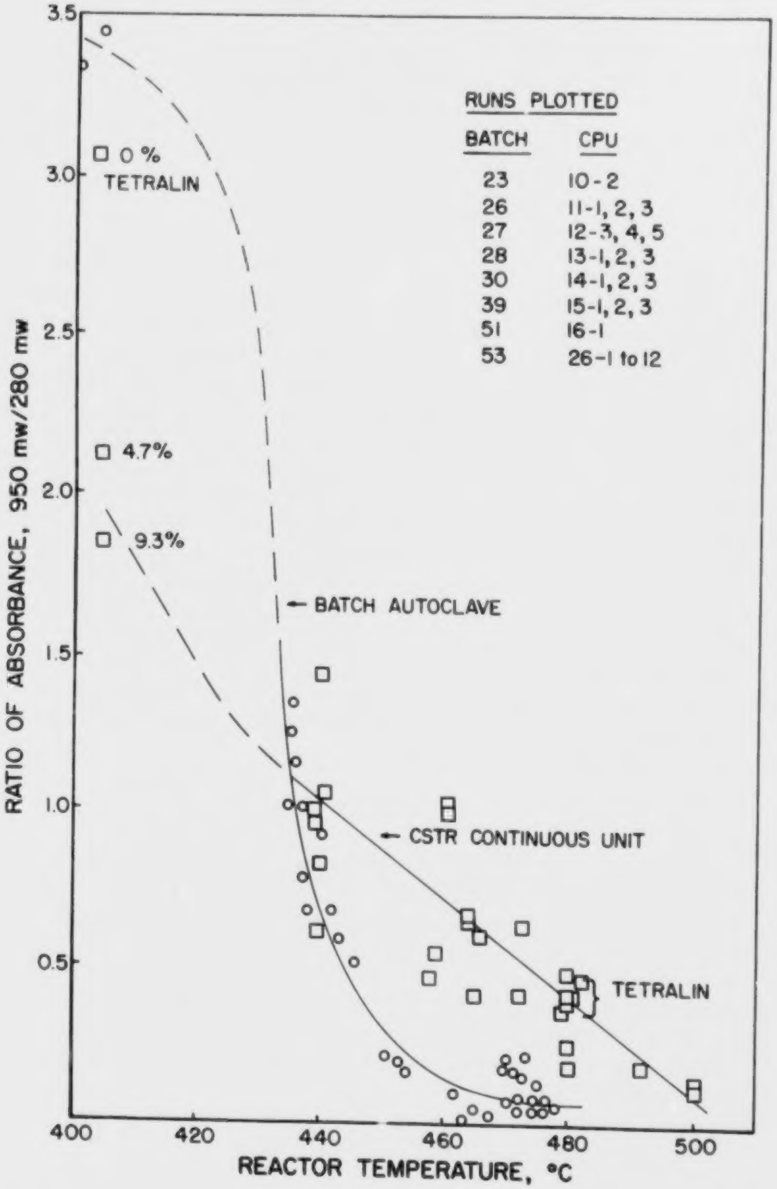

FIGURE 9 Ratio of U.V. absorbance at 254 nm of 950 MW to 250 MW material versus reactor temperature for reaction times under 30 minutes.

PB82-211640

FOUR-HOUR ALGAL BIOASSAYS FOR ASSESSING THE TOXICITY OF COAL-DERIVED MATERIALS

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ABSTRACT

As part of an overall program to assess the potential environmental hazards of coal conversion technologies, researchers at Oak Ridge National Laboratory have developed a rapid bioassay to measure the effects of coal-derived materials on algal photosynthesis. Algal cultures or natural algal communities are exposed to the test materials for four hours. Photosynthesis is determined by the ^{14}C-bicarbonate method during the final two hours of exposure and compared with controls for a measure of toxicity. In bioassays with individual aromatic compounds, quinones and aromatic amines were found to be particularly toxic to algae; azaarenes and thiophenes were the least toxic classes tested. Experiments with the water soluble fractions (WSFs) of more than twenty natural and synthetic oils showed that coal liquefaction products are considerably more toxic than petroleum products; shale oils are intermediate in toxicity. Further studies with particular subfractions of several WSFs have identified ether-soluble bases as the major contributors to the toxicity of coal-derived oils. The four-hour photosynthesis test has several advantages over the Algal Assay Bottle Test for measuring the toxicity of complex materials.

INTRODUCTION

Environmental scientists at Oak Ridge National Laboratory are engaged in a broad program of research concerning the environmental fate and effects of products and wastes from coal liquefaction and gasification processes. The algal bioassays described in this report are included in the environmental effects research program because algae are the dominant primary producers in most lentic (non-flowing) aquatic ecosystems. Since algae are at the base of most lentic food webs, changes in the productivity or structure of the algal community could have important consequences for the rest of the ecosystem.

ALGAL PHOTOSYNTHESIS TEST

The algal bioassay was developed to meet two principal objectives:

1. Rapid screening of coal-derived materials (liquid products and fractions thereof, solid waste leachates, and wastewaters) for acute toxicity to freshwater algae.
2. Determination of suitable concentrations for further effects testing.

The primary function of the bioassay is to compare the toxicities of different materials. Comparisons are based on a simple toxicological response: short-term inhibition of algal photosynthesis. The bioassay can be used with any algal culture or natural algal community. In our experiments, the test organisms are *Selenastrum capricornutum*, a green alga, and *Microcystis aeruginosa*, a non-nitrogen-fixing blue-green alga. These are two of the species recommended for the Environmental Protection Agency's Algal Assay Bottle Test (AA:BT) (9). The medium and growth conditions for these cultures are specified by Miller et al. (9).

Aliquots (18 or 20 ml) of the cultures are distributed to 20 x 120 mm screw-capped culture tubes. The test material is then added in aqueous (2 ml) or acetone (0.1 ml) solution, and the cultures are placed under a bank of fluorescent and incandescent lights (1700 μWatts cm^{-2} irradiation between 400 and 700 nm) in an environmental chamber at 24°C. The cultures are pre-incubated for 2 hr before photosynthesis measurement is begun. Pre-incubation is included in the procedure because some materials, such as naphthalene (12), do not inhibit algal photosynthesis immediately but require 60-90 min to produce a full effect. After pre-incubation, cultures are spiked with 0.01 ml (0.1 μCi) of a ^{14}C-labelled sodium bicarbonate solution and incubated for another 2 hr. Photosynthesis is stopped by the addition of 0.05 ml formalin at the end of the incubation period. The amount of ^{14}C that has been converted to organic form by photosynthesis is determined by acidifying 5-ml samples with 0.1 ml of 0.1 *N* HCl, bubbling with air for 20 min to remove inorganic ^{14}C, and measuring the remaining activity by liquid scintillation spectrometry (6). A set of controls is included in each experiment, and the response of the algae to the test material is assessed by comparing treated cultures with controls. When test materials are added in acetone solution, a set of acetone controls is also run; acetone has never had a detectable effect on photosynthesis in our experiments.

Some of the results presented below are expressed as EC_{20} values. The EC_{20} is the concentration of test material causing 20% inhibition of photosynthesis (i.e., photosynthesis is 80% of controls), as determined by interpolation on a plot of photosynthesis vs. log concentration.

STUDIES WITH SINGLE COMPOUNDS

In the course of developing the photosynthesis test, we compared the toxicity to *S. capricornutum* and *M. aeruginosa* of more than 30 aromatic compounds, including unsubstituted and methylated hydrocarbons, phenols, quinones, amines, azaarenes, and thiophenes (6). Some of the results for *M. aeruginosa* are shown in Figure 1. For each class of compounds except quinones, toxicity increased with increasing number of aromatic rings. Quinones were by far the most toxic class of compounds tested; amines were the next most toxic. Thiophenes and azaarenes were the least toxic. These generalizations also applied to *S. capricornutum* (6). Information on the relative toxicities of these common aromatic compounds has been useful in interpreting results of bioassays with complex coal-derived mixtures.

The toxicity of most of the single compounds and complex mixtures we have tested was approximately the same (within a factor of 5) for S. capricornutum and M. aeruginosa. The responses of both species were consistent with those of natural algal communities collected from a shallow pond (Table 1). Eight aromatic compounds and three oil extracts were ranked in essentially the same order by natural communities, S. capricornutum, and M. aeruginosa.

The 4-hr algal bioassay also ranked 30 aromatic compounds in approximately the same order of toxicity as 48-hr Daphnia magna LC_{50} tests (11). The interpolated EC_{20} values for most of the aromatics tested were within one order of magnitude of the D. magna 48-hr LC_{50}'s (Figure 2). The fact that concentrations causing slight photosynthetic inhibition in algae generally caused extensive mortality in D. magna could be considered evidence that algae are less sensitive to toxicants than zooplankton (8). However, it is difficult to compare the true ecological significance of the responses of the two taxa, particularly since the bioassays were of different lengths.

STUDIES WITH COMPLEX MIXTURES

The applicability of the 4-hr algal bioassay to testing complex coal-derived materials is illustrated by experiments with the water soluble fractions (WSFs) of coal liquefaction products. We chose to test WSFs rather than whole oils because the acute toxicity of oils to aquatic organisms is generally caused by the oils' water soluble components (3,10). By testing WSFs we also avoid the dosage problems caused by the immiscibility of oils in water. We prepare WSFs by floating oil on distilled water in a 1:8 (oil:water) ratio and stirring very gently for 16 hr in the dark. The WSFs are then separated from the oils and filtered (Whatman No. 41) before testing. Dilutions are made in fresh algal growth medium (9). Test solution concentrations are expressed as percentages of full-strength WSF.

The initial objective of this work was to compare the toxicities of WSFs prepared from coal liquefaction products, shale oil products, and petroleum products. Selected dose-response curves for one oil of each type are presented in Figure 3. The petroleum-derived marine diesel fuel (DFM) was the most toxic of the petroleum products we tested, including No. 2 diesel fuel, a jet fuel, and three residual fuel oils. Crude shale oil was more toxic than petroleum DFM (Figure 3); refined shale oil products, however, were similar in toxicity to petroleum products. Coal liquefaction products were consistently more toxic to algae than shale oil or petroleum (Figure 3). The 11 unrefined coal-derived oils we tested were all toxic to S. capricornutum at WSF concentrations of 1% or less, whereas petroleum WSFs were generally nontoxic even at 10% (7).

We suspected that the difference in toxicities of coal-derived and petroleum WSFs was due to the abundance of phenols and aromatic amines in coal liquefaction products. To investigate this possibility, we fractionated the WSFs of several liquefaction products into ether-soluble acid, ether-soluble base, and neutral components, and tested each subfraction at one-tenth of its concentration in the original, full-strength WSF. A consistent pattern emerged (Figure 4). The ether-soluble bases were the most toxic subfraction in nearly every case. The neutral subfractions, with one exception, were the least toxic to both species. The effects of ether-soluble acids varied considerably among oils and between species. Our conclusion, based on these results, was that the bases (mostly aromatic amines) and, secondarily, the acids (mostly phenols), were the major toxic constituents of WSFs of coal liquefaction products.

COMPARISONS WITH ALGAL ASSAY BOTTLE TEST

The Algal Assay Bottle Test (AA:BT) (9), originally developed during the late 1960's to study the causes of eutrophication in lakes, has recently been applied to the testing of toxic materials. The Environmental Protection Agency is considering the AA:BT as a standard method for testing chemicals regulated under the Toxic Substances Control Act (2), and a modified version of the AA:BT appears in the latest edition of *Standard Methods for the Examination of Water and Waste Water* (1). Since widespread adoption of the AA:BT appears imminent, we have begun testing selected materials by this method for comparison with the 4-hr photosynthesis test.

Briefly, the AA:BT involves inoculating test solutions with a small number of algal cells, incubating the cultures for 14 days, and measuring the final yield of algal biomass. Our AA:BT experiments were conducted as prescribed by Miller et al. (9) except that the culture flasks were capped with foil to reduce the loss of test compounds by volatilization. Algal biomass was determined after 14 days of growth by vacuum-filtering 3 20-ml aliquots from each culture onto pre-rinsed, dried, tared, 0.4-μ Nucleopore filters; drying the filters at room temperature over anhydrous calcium sulfate; and weighing each filter on a Cahn microbalance. Net dry weights of the treated cultures were compared with dry weights of controls to determine the effect of each treatment.

The results of the first AA:BT experiments are presented in Table 2, with results of photosynthesis tests on the same compounds included for comparison. The two methods agreed closely in some cases and diverged widely in others. Neither method was consistently more sensitive than the other. The discrepancies may reflect different measured responses, different exposure times, or experimental artifacts. We are now running additional AA:BT experiments to permit more conclusive comparisons.

Because of its speed, small scale, and technical simplicity, the 4-hr photosynthesis test is more efficient than the AA:BT for testing large numbers of materials. The photosynthesis bioassay is, obviously, more rapid than the AA:BT; the entire procedure for the photosynthesis test, including preparation, exposure, and analysis, can be completed in less than 2 days. The 4-hr test takes less space in an environmental chamber than the AA:BT, so more treatments can be included in a single experiment and thus can be compared with a single set of controls. Measurement of photosynthesis by the ^{14}C method is no more technically demanding than measurement of biovolume with an electronic particle counter, and much less tedious than direct determination of dry weight.

The AA:BT is not easily applied to testing volatile or unstable materials, including most of the single compounds and complex mixtures of concern to coal conversion. This is because the concentration and chemical composition of such materials may change drastically during a 14-day experiment. Photolysis, hydrolysis, and biodegradation of the test material are unavoidable. Volatilization can be reduced by sealing the flasks, as we did in our AA:BT experiments, but this also shuts off the CO_2 supply to the algae and can prevent the cultures from reaching their normal maximum biomass (4).

Not only the test substance, but also the exposure conditions, may change during an AA:BT experiment. "Bottle effects", that is, effects of enclosure on an algal population or community, become more pronounced with increasing incubation time. As an algal culture ages, changes occur in pH and other chemical variables, nutrient levels decline, algal metabolites accumulate in the medium, bacteria develop on the walls of the vessel, and the algae undergo a sequence of profound physiological changes (5). Most of these factors can affect the response of a test organism to a chemical.

An objective of most standardized bioassay procedures is to control the test substance, the exposure conditions, and the physiological state of the test organism as closely as possible. Since all of these variables change continuously in an AA:BT experiment, unequivocal interpretation of the results is practically impossible. For example, the AA:BT cannot be expected to produce a valid dose-response relationship if the dose cannot be unambiguously defined. There are two ways to minimize or avoid these difficulties. One solution is to adopt continuous culture techniques (5) if long-term exposures are desired. The other strategy is to minimize the enclosure time, as we have done with the photosynthesis test.

One of the greatest challenges of environmental toxicology is relating laboratory test results to a natural ecological context. This is especially difficult with the AA:BT; the maximum yield of algal biomass is relevant to the problems of eutrophication but not to the effects of toxic substances. Algae in nature rarely experience a stationary phase comparable to the measured endpoint of the AA:BT. Even a high standing crop of algae can sustain animal populations only if the algae that are consumed are continually replaced by new production. The rate of primary production sets an upper limit to the rate of secondary production in most lentic ecosystems. Since the 4-hr photosynthesis test detects effects on algal productivity directly, if only for a short period of time, it is more ecologically meaningful than the AA:BT.

CONCLUSIONS

Because it is simple, rapid, and inexpensive, the 4-hr algal photosynthesis test has proven useful in screening large numbers of coal-related substances. The responses of *S. capricornutum* and *M. aeruginosa* were similar to those of natural algal communities in our experiments, and also correlated well with _D. magna_ acute toxicity tests. The 4-hr algal photosynthesis test is a convenient, ecologically relevant alternative to the Algal Assay Bottle Test for toxicity screening of simple and complex materials.

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TABLE 1

Acute Toxicity of Aromatic Compounds (EC_{20} in ppm) and Water Soluble Fractions (EC_{20} in %) to Algal Cultures and Natural Algal Communities

Aromatics	*Selenastrum capricornutum*	*Microcystis aeruginosa*	Natural Algal Community
Phenanthrene	0.7	0.15	0.25
1-Naphthylamine	1.7	0.22	0.23
2-Naphthol	4	0.75	1.0
Naphthalene	5.7	0.85	1.4
Acridine	1.3	3.6	3.3
Phenol	174	54	102
Quinoline	25	117	110
Benzene	144	50	250
Water Soluble Fractions			
Petroleum DFM	13		4.5
Coal-Derived Fuel Oil	0.4	0.5	0.2
Coal-Derived Distillate	0.4	0.9	0.4

TABLE 2

Toxicity of Aromatic Compounds to Two Algae:
Comparison of Algal Photosynthesis Bioassay (APB)
and the Algal Assay Bottle Test (AA:BT)

Species	Compound	-EC_{20} (ppm)-- APB	AA:BT
S. capricornutum	acridine	1	0.5
"	naphthalene	6	30
M. aeruginosa	phenanthrene	0.2	3
"	2-naphthol	0.8	20
"	quinoline	100	7
"	acridine	4	20

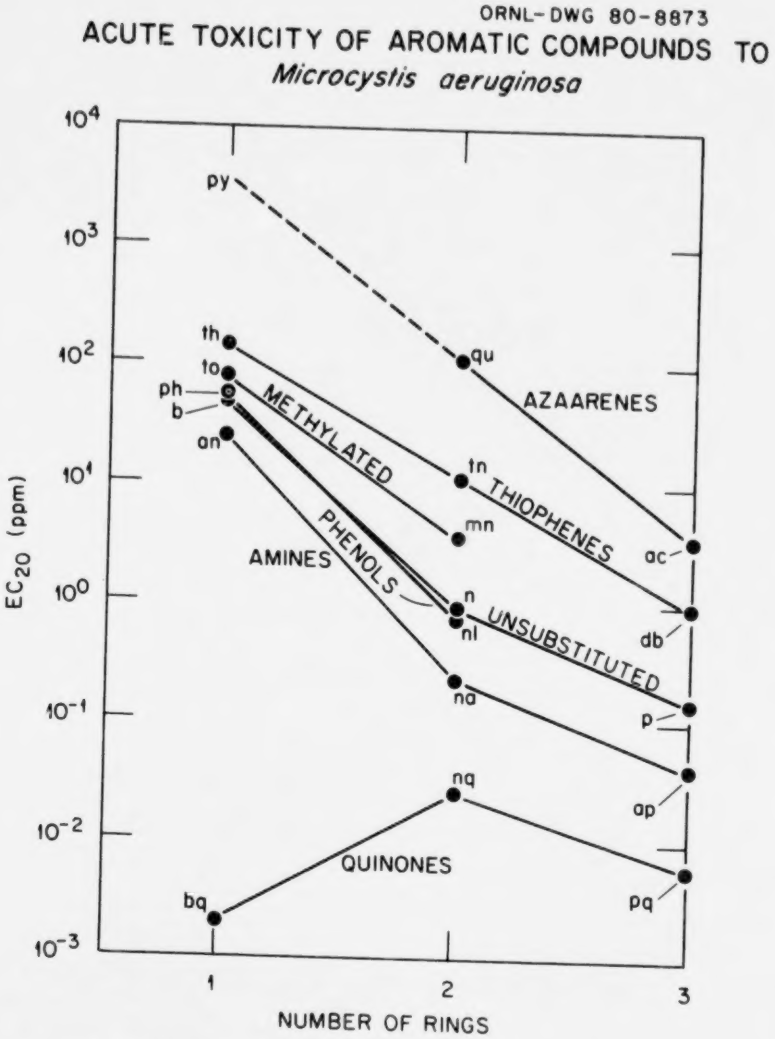

FIGURE 1 Acute toxicity of aromatic compounds to Microcystis aeruginosa. ac = acridine; an = aniline; ap = 9-aminophenanthrene; b = benzene; bq = benzoquinone; db = dibenzothiophene; mn = 1-methylnaphthalene; n = naphthalene; na = 1-naphthylamine; nl = 2-naphthol; nq = naphthoquinone; p = phenanthrene; ph = phenol; pq = phenanthraquinone; py = pyridine; qu = quinoline; th = thiophene; tn = thianaphthene; to = toluene. The line connecting pyridine and quinoline is estimated; pyridine was not toxic at 1000 ppm, the highest concentration tested.

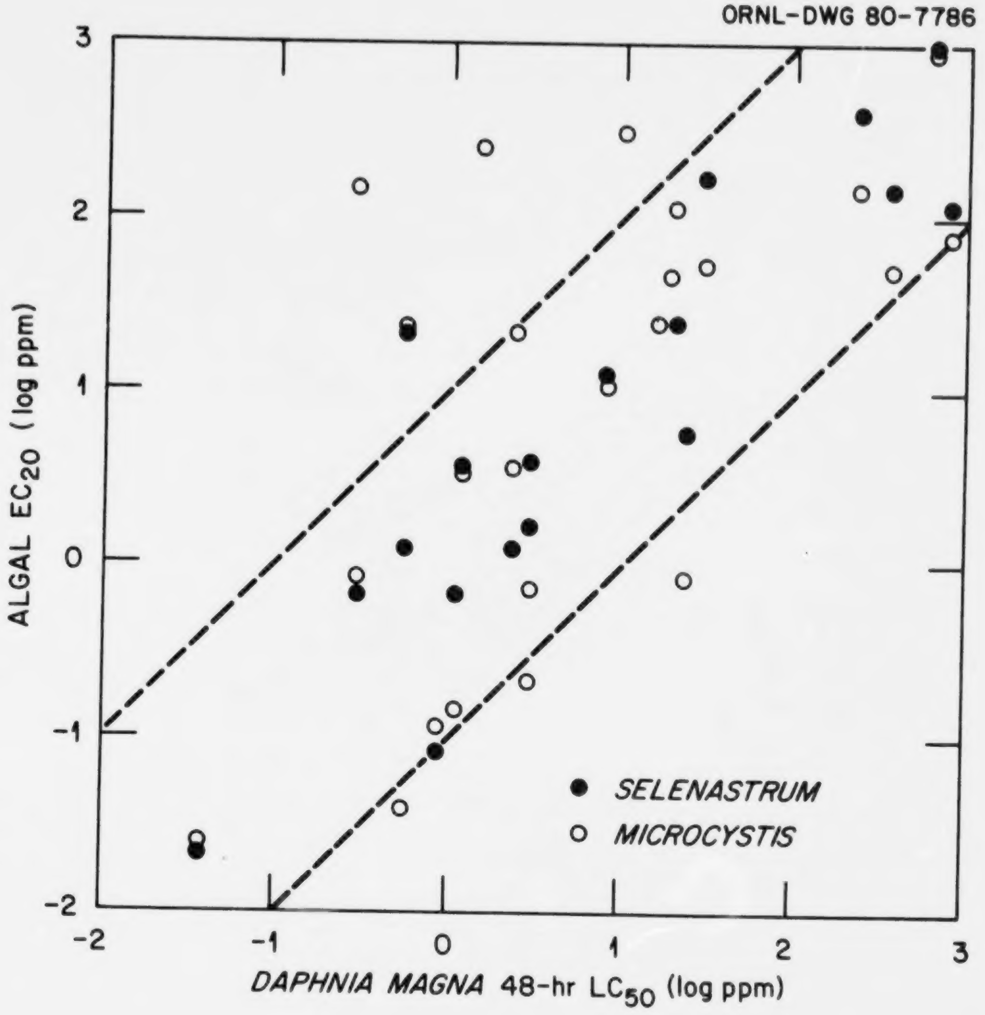

FIGURE 2 Comparison of acute toxicities of aromatic compounds to algae and to D. magna. Each point represents one compound. Broken diagonals enclose compounds for which algal EC_{20} and D. magna LC_{50} differ by less than one order of magnitude. D. magna data from Parkhurst (11).

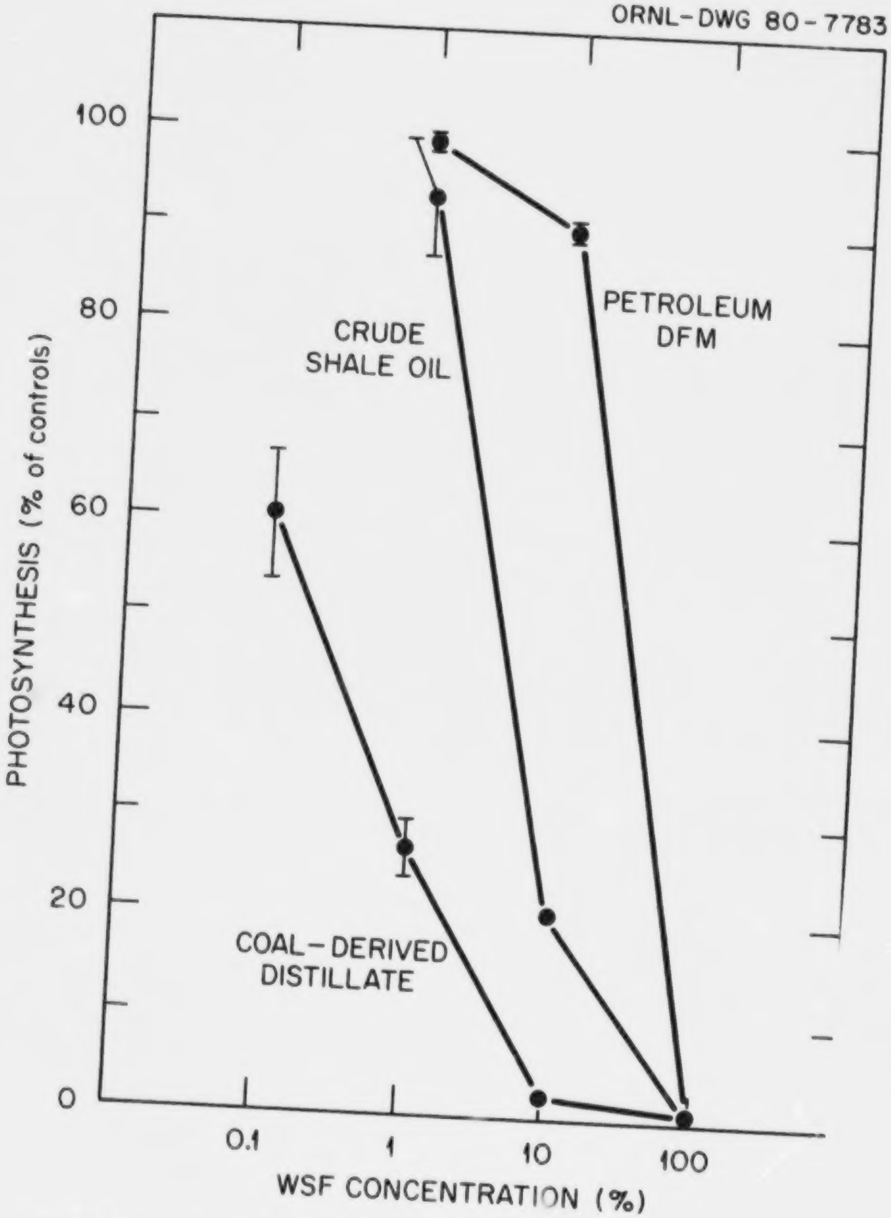

FIGURE 3 Relative photosynthesis (% of controls) of *Selenastrum capricornutum* exposed to WSFs of petroleum-derived marine diesel fuel (DFM), crude shale oil, and a coal-derived distillate oil. Error bars indicate ± 1 S.D.; they are omitted if they would not extend beyond the data point.

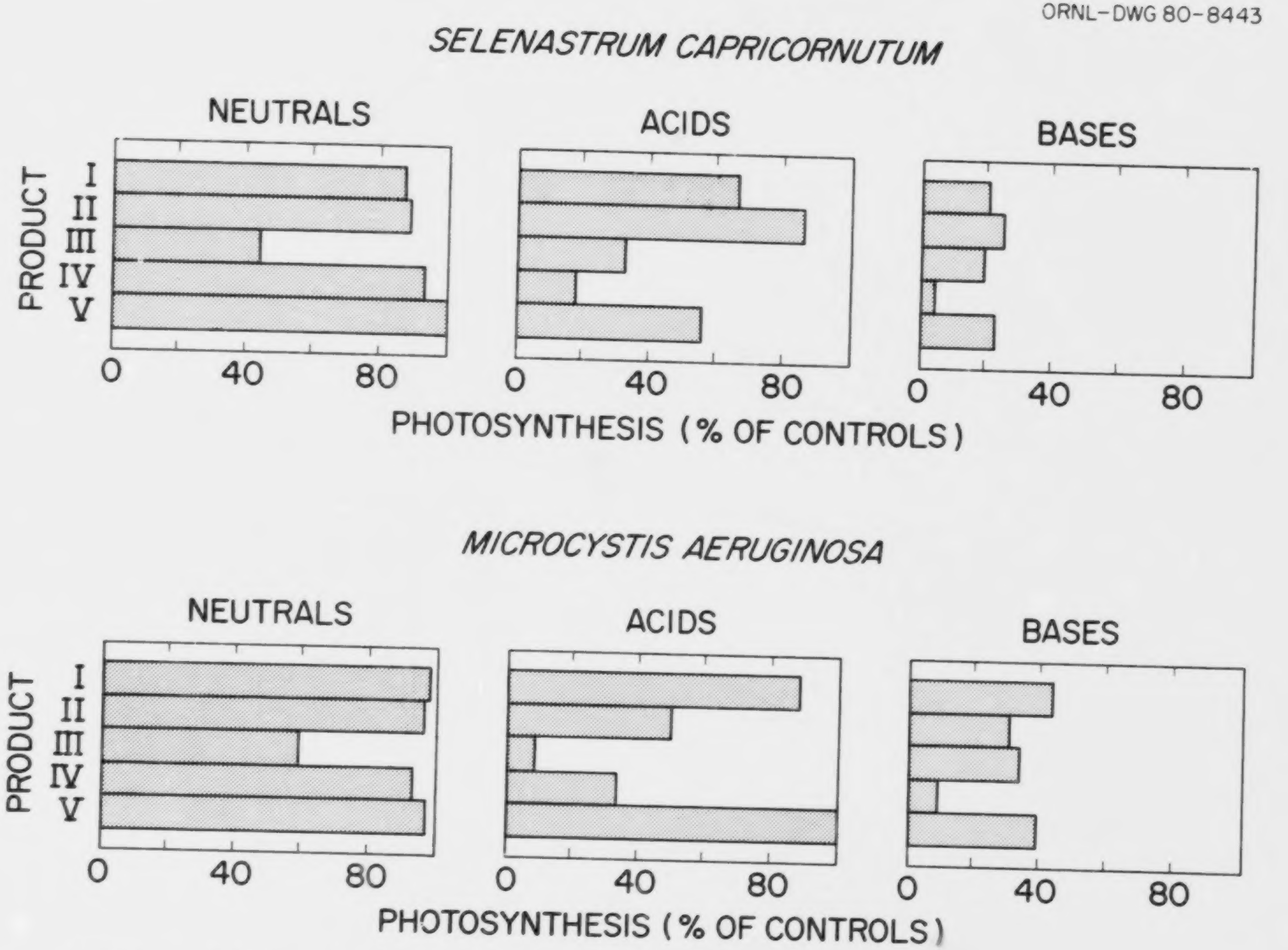

FIGURE 4 Relative photosynthesis (% of controls) of Selenastrum capricornutum and Microcystis aeruginosa exposed to ether-soluble acids, ether-soluble bases, and neutral components of water soluble fractions of 5 coal liquefaction products. Each subfraction was tested at one-tenth of its concentration in full-strength WSF.

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DIRECT DETERMINATION OF POLYNUCLEAR AROMATIC HYDROCARBONS IN COAL LIQUIDS AND SHALE OIL

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Elaborate analytical methodologies are usually utilized to determine the carcinogenic benz[a]pyrene and other potentially carcinogenic polynuclear aromatic hydrocarbons (PAHs) present in coal liquifaction products and shale oil, the liquid fuels of the future. Existing methodologies require that individual compounds or a small group of compounds be isolated by utilizing high performance liquid chromatography (1), capillary column gas chromatography (2, 3) or thin layer chromatography (4). Detection and quantitation of the isolated PAH compounds is usually performed by mass spectrometry or luminescence spectrometry.

Coal liquids and shale oil are very complex mixtures of organic compounds and their analysis by conventional techniques is relatively difficult. In contrast, laser excited Shpol'skii effect spectroscopy (LESS) is a simple and less elaborate approach that has been utilized for the direct determination of benz[a]pyrene and other PAHs in SRC II, a coal liquid and a sample of shale oil. The determinations are accomplished without prior isolation of the compounds.

For the analysis of complex mixtures of PAHs, the spectral resolution in the fluorescence spectra can be enhanced by adopting low temperature luminescence techniques. Currently, the most promising techniques are Shpol'skii effect spectroscopy in n-alkane hosts (5, 6), fluorescence line narrowing spectroscopy (FLNS) in organic glasses (7, 8) and matrix isolation (MI) spectroscopy in a frozen nitrogen matrix (9, 10). Typical bandwidths (full width at half maximum, FWHM) in the electronic spectra of PAHs in organic glasses. frozen N_2 and n-alkane matrices are ~ 200 cm^{-1}, ~ 100 cm^{-1}, and ~ 10 cm^{-1}, respectively. In FLNS and MI, the resolution in the luminescence spectra is achieved by the application of narrow bandwidth tunable dye lasers. The inherent, relatively narrow bandwidths in the electronic spectra of PAHs in n-alkane solvents is a significant advantage in achieving high resolution luminescence spectra. The resolution can be further enhanced in Shpol'skii effect spectroscopy, as tunable narrow bandwidth (~ 0.02 nm at 400 nm) dye lasers can be utilized to selectively excite any given compound present in a highly complex mixture of compounds. Thus, laser excited Shpol'skii effect spectroscopy (LESS) of PAHs in n-octane, frozen to a solid at 15K, has been utilized to achieve the following: (1) site selected luminescence of a PAH compound, (2) selectively excited luminescence of any individual alkylated benz[a]anthracene present in a mixture of multialkylated benz[a]anthracenes and (3) quantitate selected PAHs including benz[a]pyrene in diluted samples of SRC II and shale oil.

The apparatus for experimental procedures are described in a communication submitted for publication in Analytical Chemistry (11). A comparison of the data obtained by LESS with that obtained by the NBS and by other techniques at the Ames Laboratory is also presented in the communication.

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SYNCHRONOUS FLUORESCENCE AND PHOSPHORESCENCE AT ROOM TEMPERATURE FOR LEVELS 1 AND 2 ORGANIC ANALYSIS

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ABSTRACT

By simple and rapid synchronous luminescence techniques, analysis was carried out on thirteen polynuclear components in a liquid chromatography fractionated XAD-2 extract from Source Assessment Sampling System runs. This same sample was used in an interlaboratory evaluation of current Environmental Protection Agency Level 1 organic analysis procedures. Comparative results show that synchronous fluorescence and room temperature phosphorescence provide data that are more than adequate for Level 1. These techniques should also be seriously considered in the development of Level 2 organic analysis procedures. An example is given of the use of luminescence for rapid screening of an unfractionated coal liquefaction product for a major PNA component.

INTRODUCTION

The interlaboratory examination of combined XAD-2 extracts collected by Source Assessment Sampling Systems (SASS) is part of a study (1) to verify the efficacy of the analytical scheme outlined in the *Industrial Environmental Research Laboratory - Research Triangle Park Procedures Manual: Level 1 Environmental Assessment* (2). The study is coordinated by the Industrial Environmental Research Laboratory - Research Triangle Park (IERL-RTP). Our contribution to this study is to gauge how well certain rapid, easy to use, and cost-effective luminescence techniques can be employed for the analysis of polynuclear aromatic (PNA) compounds.

By demonstrating success we hope to "popularize" the luminescence techniques so they will be accepted into the tiered or phased approach to analytical investigation (Levels 1 and 2) that is advocated by the EPA (3).

Two luminescence techniques are considered; synchronous luminescence (SL) spectroscopy of solutions at room temperature (4) and room temperature phosphorimetry (RTP) (5).

METHODOLOGY

Synchronous Fluorescence (SF) and Synchronous Phosphorescence (SP)

In conventional luminescence spectroscopy, either the excitation (λ_{ex}) or the emission wavelength (λ_{em}) remains fixed. With synchronous luminescence, the luminescence signal is recorded while λ_{ex} and λ_{em} are scanned simultaneously at a fixed wavelength interval, $\Delta\lambda$, between the two.

The principal advantage of this technique is improved selectivity. This results from a narrowing of a targeted spectral band, or bands, and a reduction or elimination of spectral emissions from other compounds that would otherwise cause interference effects. The theory and selection of appropriate values of $\Delta\lambda$ have been dealt with previously (4). The conventional and synchronous fluorescence spectra of a synthetic mixture of five PNA compounds (4) are shown in Figure 1 for the purpose of familiarizing the reader with the degree of spectral simplification that is possible.

The concept of synchronous excitation is applicable to phosphorimetry (5), as well as fluorimetry (4). In the case of SF the Stokes' shift determines the optimum value of $\Delta\lambda$ which is often set at 3 nm (4). For SP it is the singlet-triplet energy difference that determines the optimum value of $\Delta\lambda$ (6); for most PNA compounds $\Delta\lambda$ is the range of 100 to 300 nm.

A strong point of the synchronous technique is the simplicity of instrumentation. The spectrometer employed for synchronous measurements is often the same instrument used for measurement of conventional fixed-excitation luminescence. Several instruments with interlocking capability of the two monochromators, as a standard feature, are commercially available. Even with an already existing spectrometer that does not have the interlocking feature, a simple and inexpensive switch can be added for synchronous scanning.

In some cases, further enhancement in selectivity is achieved by derivatization of the luminescence signal output (7). This method, referred to as derivative luminescence spectroscopy, consists of measuring the first, second, or higher derivative of a spectrum with respect to wavelength. It is used mainly to enhance minor spectral features superimposed on a more intense background. In the second-derivative (d^2) mode, where one measures the curvature of a peak, broad bands are suppressed while sharp bands are intensified. An illustration of this effect, for the RTP spectra of fluorene (7), is illustrated in Figure 2.

The application of synchronous luminescence to the analysis of complex organic mixtures is not without its limitations and potential pitfalls. The user needs to be aware of these. The range of accessible compounds is limited. Some PNA compounds fluoresce or phosphoresce too weakly to make their measurement practicable. Alternately they absorb or luminesce at wavelengths not easily accessible.

Sample dilution is often necessary to avoid anomalies caused by inner filter effects, energy transfer and/or quenching. One needs to work within a range where the response varies linearly with concentration. The dilution necessary to achieve this, however, can reduce the concentration of trace, or weakly luminescing constituents below their limits of detection. Discussions of these problems, as they effect analyses by synchronous luminescence, have appeared recently (8,9).

Room Temperature Phosphorimetry (RTP)

The relatively new technique of RTP is based on the detection of phosphorescence from organic compounds adsorbed on solid substrates at room temperature. This type of phosphorimetry has been reviewed (10).

Normally the solid substrate is filter paper. The sample solution is spotted on the filter paper and after drying, phosphorescence is measured by a spectrofluorimeter equipped with a rotating phosphoroscope. The simplicity of the procedure is a distinct advantage. Expanded experimental details can be found in reference 11.

To improve the sensitivity and lower the detection limit to the nanogram or subnanogram range, one resorts to the use of heavy-atom perturbers. For PNA compounds, a variety of heavy atom salts, such as cesium iodide, sodium bromide, lead acetate and thallium acetate, are very efficient in enhancing the phosphorescence quantum yields of most PNA compounds in a selective manner (12). The filter paper can be pretreated with the perturber or the heavy atom salt can be added after spotting the paper with the PNA solution.

The fact that these operations are rapidly and easily carried out, makes the technique of RTP attractive for the screening of large numbers of PNA bearing samples. The ease with which automated sampling and analysis (11) could be carried out is also attractive. All of these features are attractive in the context of Level 1 analysis.

SAMPLES

As part of the interlaboratory study to evaluate Level 1 organic analysis (1), we were provided with two samples. The first sample was an artificial mixture of 16 compounds in methylene chloride, sample number 5 in Table 15 of reference 1. The real-life sample was pooled XAD-2 resin extract from three SASS train runs. The LC fraction in methylene chloride was designated I-TX-3 (1). The composition of this sample was determined by the collaborators of the interlaboratory study, including ourselves.

A third sample to be mentioned briefly is a coal liquid product from the Synthoil coal desulfurization process. This material was dissolved in methylene chloride and examined by RTP without any fractionation.

RESULTS AND DISCUSSION

Artificial Mixture

This sample was used to check the efficacy of the d^2 - SF technique. The identity and quantity of the 16 aliphatic and aromatic compounds that were present are listed in Table 1. By SF, phenol and four of the PNA compounds could be detected readily. The measured quantities are in good agreement with the actual concentrations (13). The aliphatic compounds are not susceptible to analysis using this technique. Quinoline fluoresces weakly but can be detected with high sensitivity using RTP (12). For the five compounds that could be detected, the analysis is accurate enough for Level 2 (14).

SASS Sample

After 1000-fold sample dilution, thirteen and ten PNA compounds could be quantified by SF and RTP, respectively. Quantitative analysis yielded the results in Table 2 (13).

The four compounds present in relatively high concentrations ($\sim 10^{-6}$ M) were phenanthrene, fluoranthene, chrysene and pyrene. Trace constituents ($\sim 10^{-7}$ to 10^{-8} M) were benzo[e]pyrene, benzo[a]pyrene, 2,3-benzofluorene, 1,2,5,6-dibenzanthracene, dibenzothiophene, perylene, fluorene, anthracene and tetracene.

It is worthwhile to note the sometimes complementary nature of SF and RTP. On the other hand benzo[e]pyrene, dibenzothiophene and phenanthrene were measured with better sensitivity and accuracy by RTP, while anthracene, perylene and tetracene were detected only by SF.

Data quoted with two significant figures, e.g. fluorene, are sufficiently accurate for Level 2. The standard deviation on these data is generally within ± 30%. The less accurate results listed in Table 2 are still acceptable for the ± 300% requirement for Level 1. These data have been reduced to a relative decade scale in Table 3. This enables the data to be compared to low resolution mass spectroscopy (LRMS) data obtained by another laboratory for Level 1 analysis. At this low level of accuracy the degree of matching is good.

To illustrate the type of spectral specificity that can be obtained, Figures 3 and 4 show RTP spectra of the SASS sample using two different excitation wavelengths and two different heavy atoms. The excitations at 365 and 343 nm are optimum for fluoranthene and pyrene, respectively. The degree of compound specificity is highlighted by inclusion of the spectra of pure pyrene and pure fluoranthene. These spectra provide convincing evidence for the unambiguous identification of major PNA components in real-life samples using RTP.

The analytical curves (log intensity vs log concentration) for fluoranthene and pyrene in Figure 5 show that linearity of response is possible over three decades of concentration.

Synthoil

Some data for Synthoil are given to show the possibilities of using synchronous luminescence to screen a completely unfractionated sample for a major PNA compound.

Pyrene is the major PNA compound in this very complex sample in a concentration of 4300 ppm (15). The RTP spectrum of the unfractionated Synthoil using lead acetate heavy atom perturber, is shown in Figure 6. Comparison with the RTP spectrum of pure pyrene indicates that a rough quantitative analysis could easily be made. The spotting on filter paper, the drying, and the recording of the spectrum took only a few minutes. The technique, therefore, can provide a means for true rapid screening of a major PNA compound, or compounds.

PROGNOSIS

The two techniques of synchronous luminescence and room temperature phosphorescence are demonstrably useful for quickly characterizing PNA bearing, real-life samples. Without any fractionation, it is possible to measure a major PNA component, such as pyrene in Synthoil, within a few minutes.

After coarse fractionation, the quantitative analysis can be extended to include a dozen or more PNA compounds. The results from synchronous luninescence can clearly reinforce or expand the knowledge forthcoming from LRMS and infrared, the two other techniques that are the basis of Level 1 organic analysis.

There are additional advantages. Once calibration curves have been constructed, the analyses are simple to conduct, rapid, inexpensive and can be performed with commercial instrumentation. The methodology is also amenable to automation.

We recommend that the synchronous luminescence techniques be seriously considered for incorporation in the EPA/IERL-RTP phased approach to sampling and analysis for environmental assessment.

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TABLE 1

Artificial Complex Sample Quantitatively Analyzed by Second Derivative Synchronous Fluorescence

Compound	Actual concentration [mg/20ml]	Measured concentration [mg/20ml]	d^2 - SF peak [nm]
Squalene	12.7		
n-Tridecane	14.4		
Biphenyl	13.6	15 ± 3	308
Chlorobenzene	20.1		
Acenaphthene	14.0	13.7 ± 0.4	328
Chrysene	15.7	16.1 ± 1.0	368
Dihexylether	17.8		
Dinitrotoluene	20.1		
Dibenzothiophene	12.7	12.4 ± 0.6	335
Diethylphthalate	14.4		
2-Ethylhexanol	15.4		
Phenol	16.7	12.0 ± 1	285
Quinoline	12.6		
Palmitic Acid	13.3		
Stearic Acid	12.2		
Di-p-tolylsulfoxide	20.4		

TABLE 2

Concentrations of PNA Compounds in the SASS Sample Extract as Measured by Synchronous Fluorescence (SF) and Room Temperature Phosphorescence (RTP)

Compound	Concentration (M)*	
	SF	RTP
Anthracene	7.7×10^{-7}	---
Phenanthrene	$<2 \times 10^{-5}$	8×10^{-6}
Fluorene	1.2×10^{-7}	1.5×10^{-7}
Dibenzothiophene	$<4 \times 10^{-7}$	3×10^{-7}
1,2,5,6-Dibenzanthracene	$<5 \times 10^{-7}$	1×10^{-7}
2,3-Benzofluorene	3.3×10^{-7}	3×10^{-7}
Fluoranthene	1.9×10^{-6}	1.6×10^{-6}
Chrysene	3.2×10^{-6}	3.6×10^{-6}
Pyrene	3.0×10^{-6}	2.3×10^{-6}
Tetracene	1.9×10^{-8}	---
Benzo[e]pyrene	$<6 \times 10^{-6}$	4×10^{-7}
Benzo[a]pyrene	6.0×10^{-7}	$<10^{-6}$
Perylene	1.0×10^{-8}	---

---

*Measurements taken with a 1000-fold diluted solution.

TABLE 3

Relative Concentrations on a Decade Scale
of PNA Compounds in the SASS Sample

Compound	Synchronous Luminescence*	Low Resolution Mass Spectroscopy (Arthur D. Little, Inc.)
Anthracene/Phenanthrene	100	100
Pyrene	100	100
Benzofluoranthenes	---	100
Fluoranthene	100	---
Chrysene, Benzanthracene	100	100
Benzopyrenes	100	10
1,2,5,6-Dibenzanthracene	10	---
2,3-Benzofluorene	10	10
Dibenzothiophene	10	10
Fluorene	10	10
Tetracene	1	---
Perylene	1	---

Concentration extrapolated to the original sample.

* $> 1 \times 10^{-3}$ M = 100

$< 1 \times 10^{-3}$ but 1×10^{-4} M = 10

$< 1 \times 10^{-4}$ M = 1

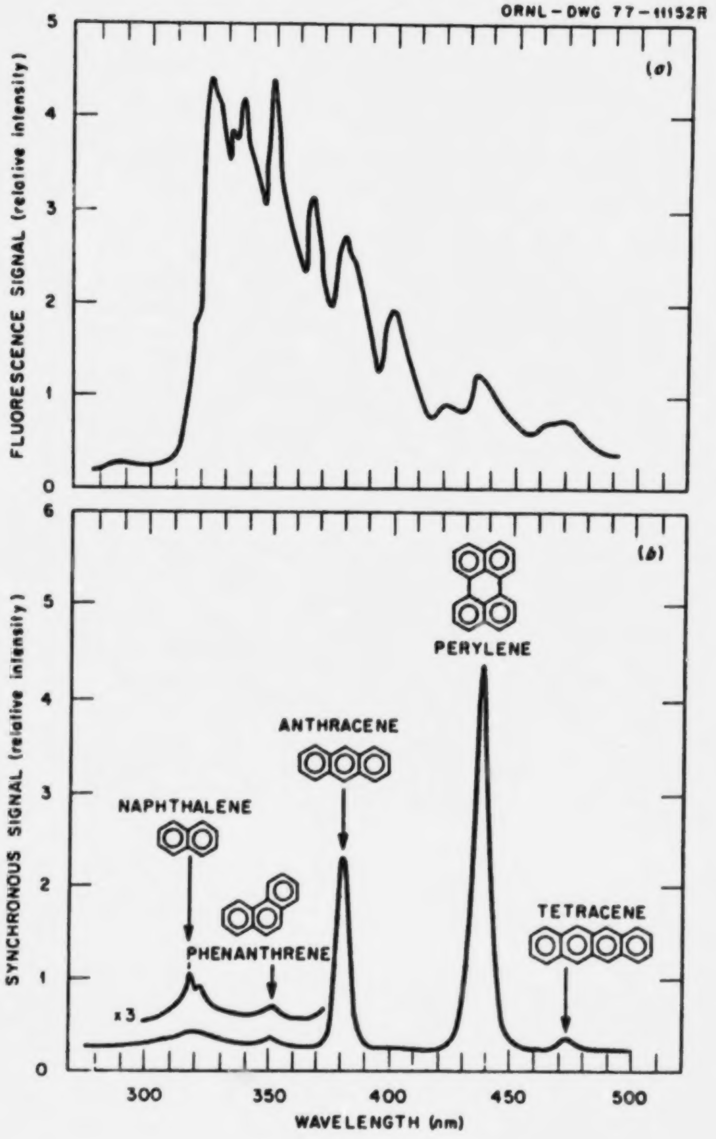

FIGURE 1 Conventional fixed-excitation and synchronous fluorescence spectra of a synthetic mixture of five PNA compounds.

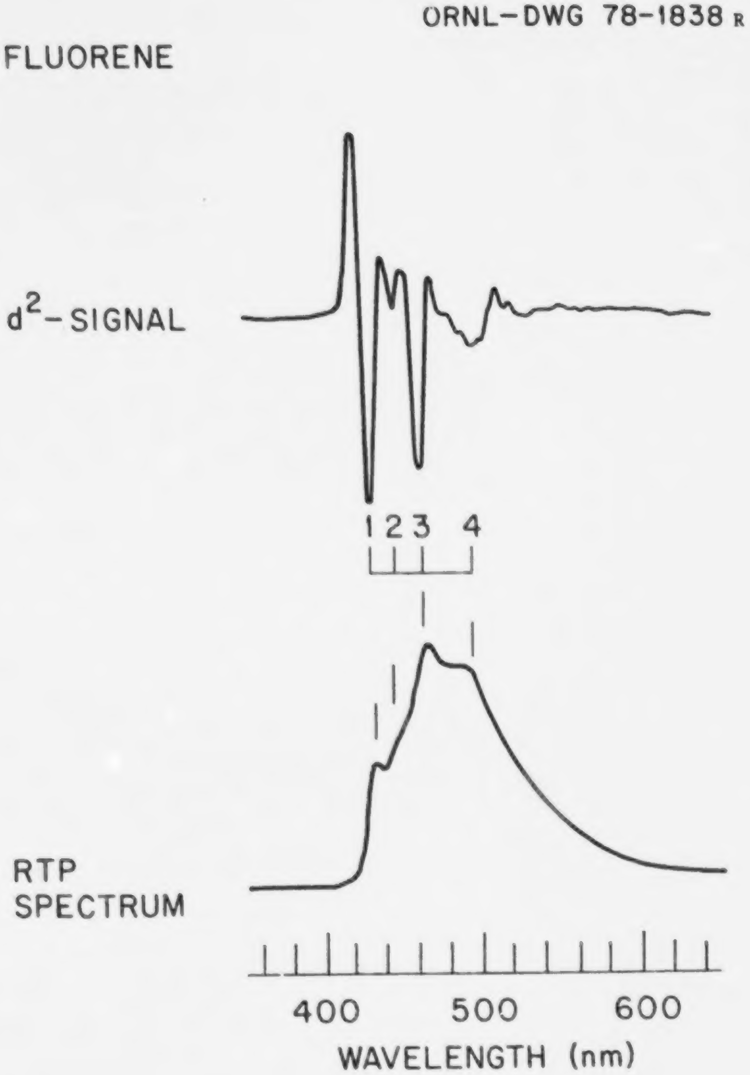

FIGURE 2 Comparison of the direct (d^0 - lower curves) and second-derivative (d^2 - upper curves) RTP spectra of fluorene.

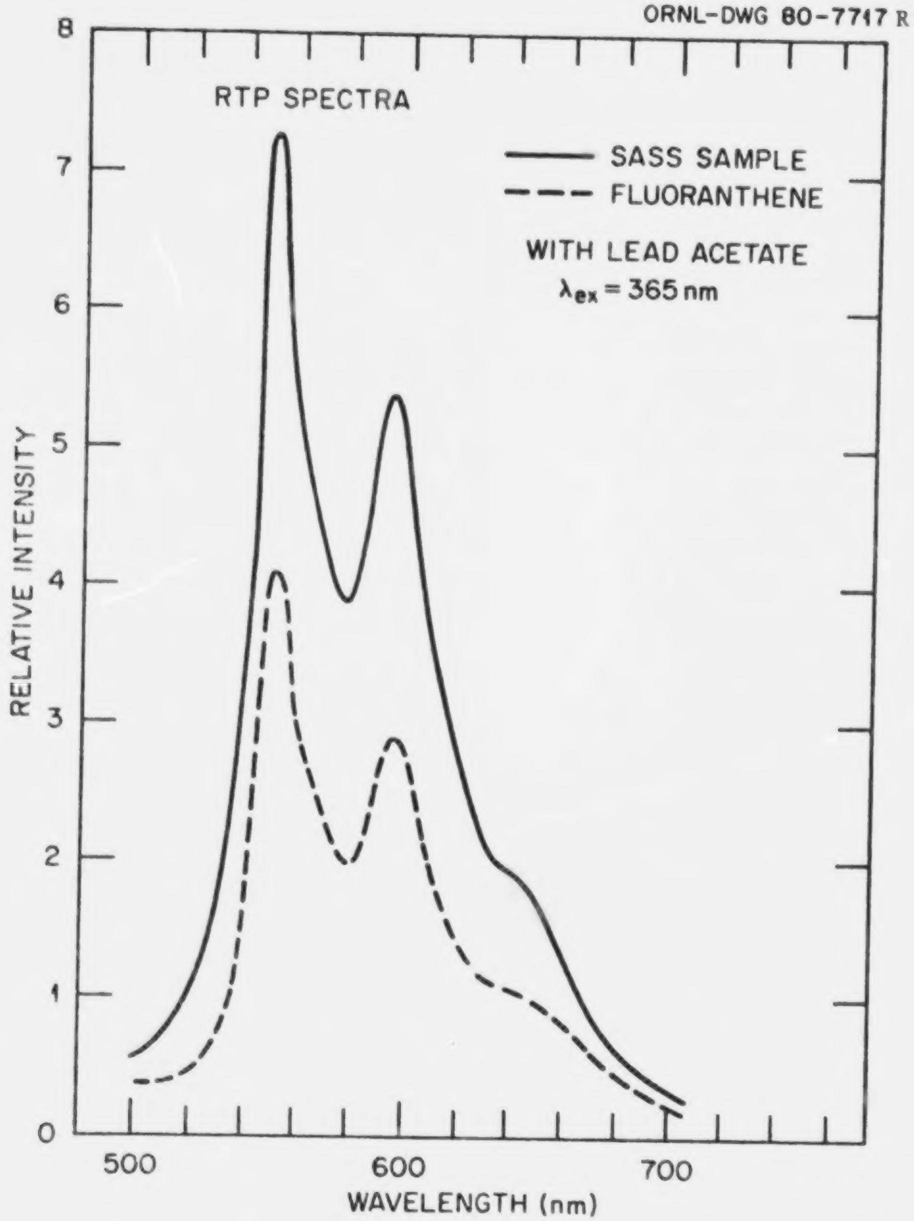

FIGURE 3 Characterization of fluoranthene in the SASS sample by room temperature phosphorimetry (solid curve: SASS sample; dashed curve: fluoranthene; λ_{ex} = 365 nm).

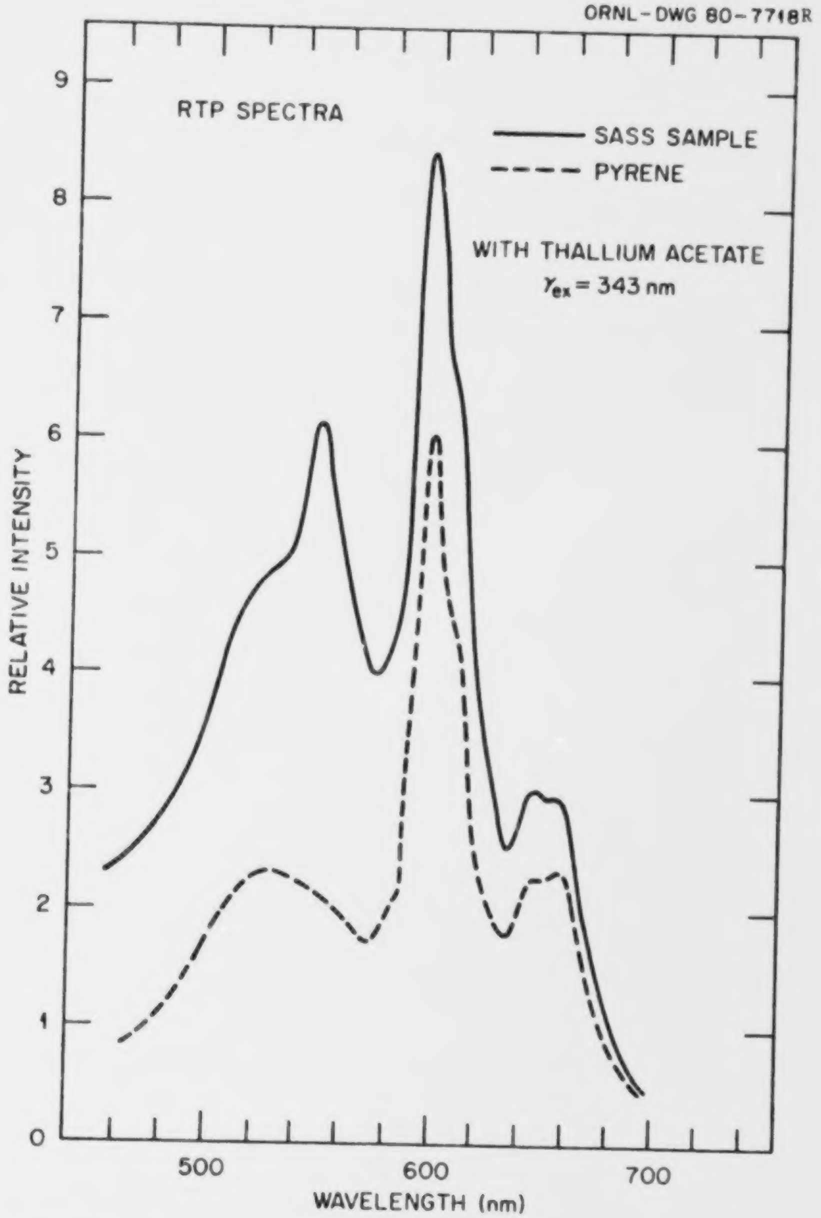

FIGURE 4 Characterization of pyrene in the SASS sample by room temperature phosphorimetry (solid curve: SASS sample; dashed curve: pyrene; λ_{ex} = 343 nm).

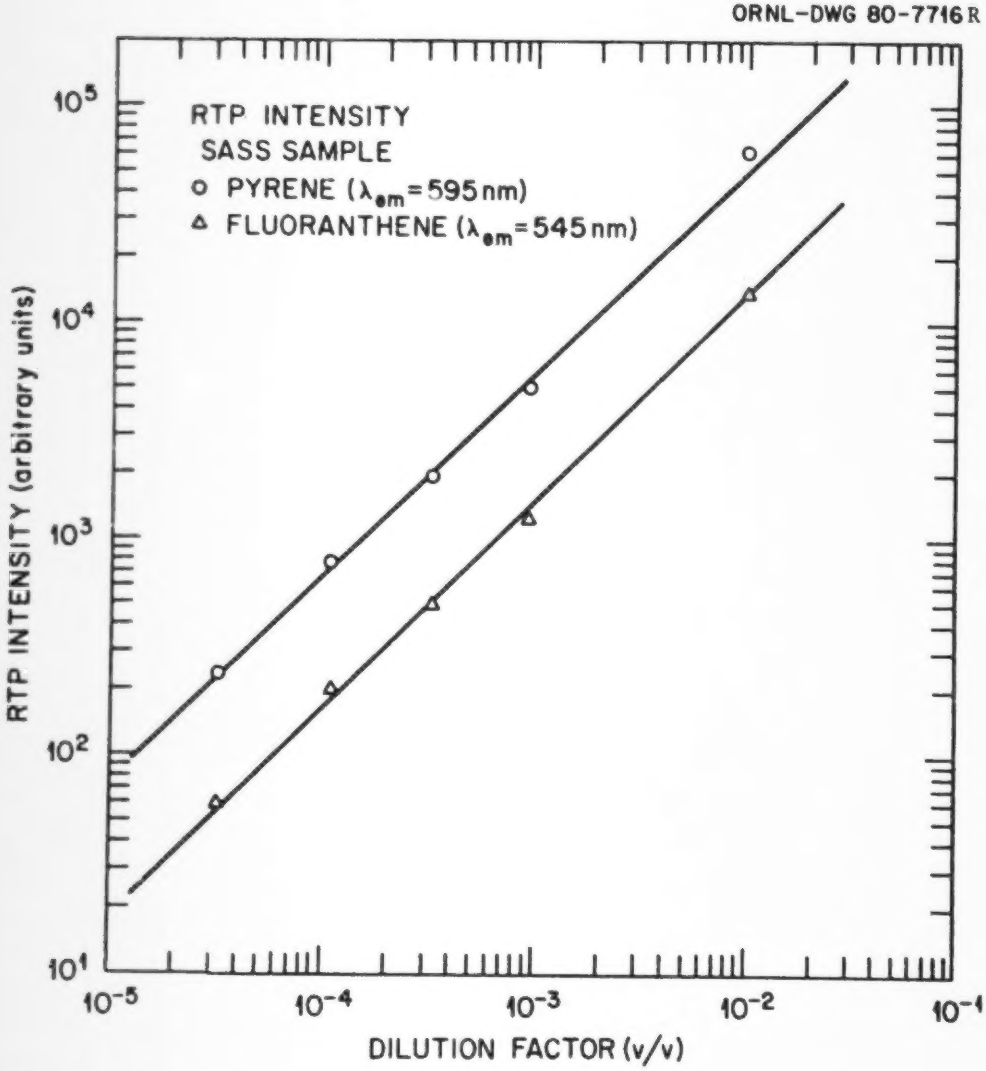

FIGURE 5 RTP analytical curves of pyrene and fluoranthene in the SASS sample.

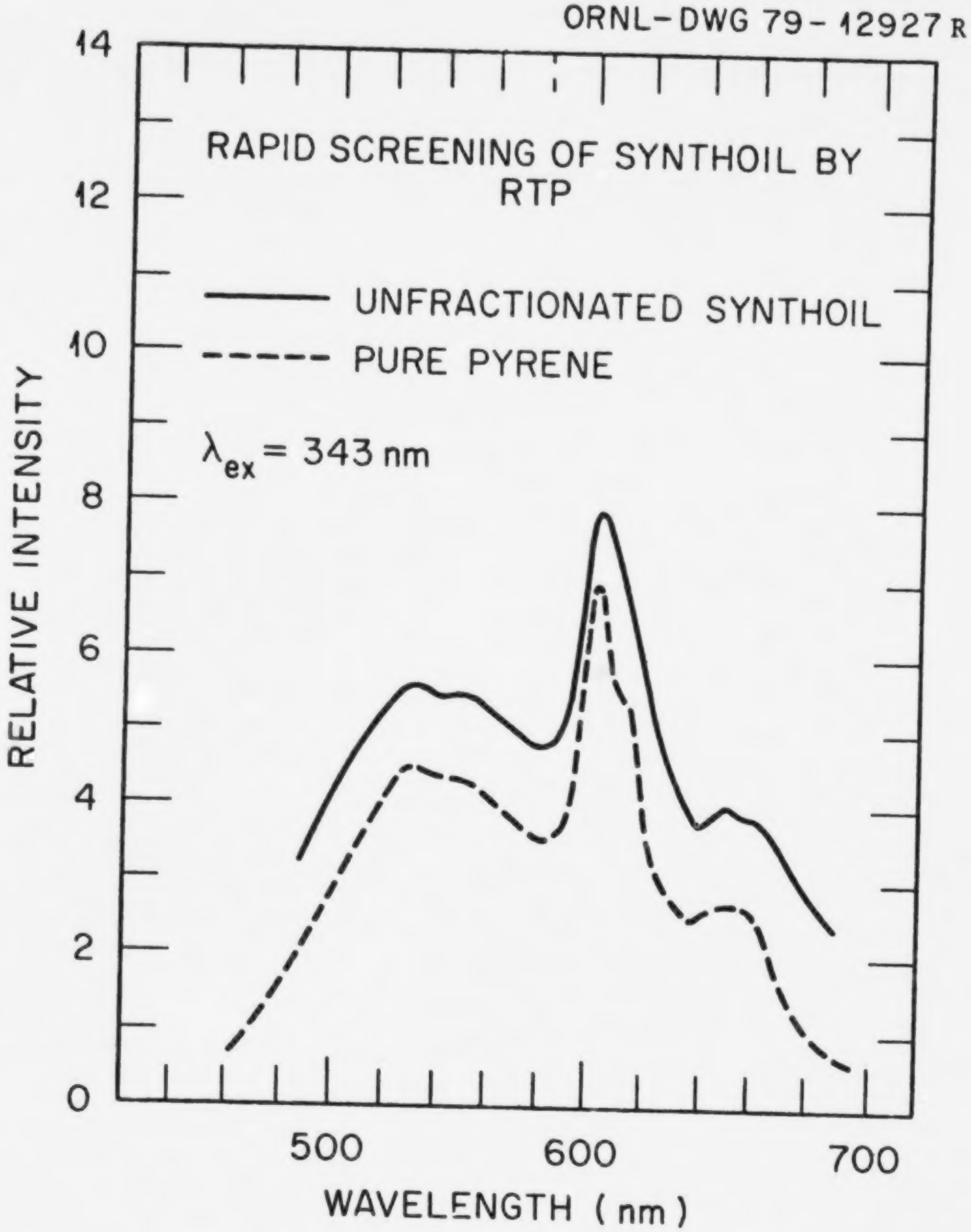

FIGURE 6 Rapid identification of pyrene in unfractionated Synthoil.

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"APPLICATION OF CONTAMINANT ENRICHMENT MODULES TO ORGANIC TRACE ANLYSIS"

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ABSTRACT

The assessment of environmental pollutants often requires the analysis of contaminants present at trace levels in complex biological matrices. These analyses are greatly facilitated by contaminant enrichment and concurrent removal of biogenic coextractives before analysis. Optimum use of sophisticated analytical systems is facilitated by effective removal of sample coextractives from contaminants. Simplification of complex residues is achieved using enrichment processes that are also specific for chemical classes. We have developed a series of modular chromatographic enrichment procedures designed to be integrated into an automated sequential system, controlled by a microprocessor, for fractionation of complex residues extracted from environmental samples. Initially removal of most coextracted biogenic compounds takes place using gel permeation chromatography (GPC), and then acidic contaminants in the GPC effluent are retained by cesium silicate. Another module, carbon dispersed on urethane foam or on glass fibers, is used to retain planar aromatics. The unretained neutrals and basic contaminants are collected and further fractionated with a variety of adsorbents. These procedures have enabled detection of chlorinated dibenzofurans, terpenes, naphthalenes, and phenolics in addition to the common organochlorine pollutants in fish samples by electron capture gas chromatography (EC-GC), negative ion mass spectrometry (NI-MS), and conventional GC/MS.

INTRODUCTION

Complex multiclass residues are usually encountered in the analysis of environmental samples for organic contaminants. Analyses of this type can be facilitated by a comprehensive approach, including fractionation of contaminants by class. In an effort to provide such a procedure, we have developed and tested three modular chromatographic procedures: gel permeation chromatography (GPC); cesium silicate chromatography (CsSC); and dispersed-carbon chromatography.

These modules were designed to achieve an integrated separation process suitable for automation. Space and manpower limitations encountered in many laboratories increase the need for automated sample cleanup and analysis. Improved routine purification capabilities made possible by chromatographic separation of chemical classes broadens the scope of contaminants amenable to analysis.

Two factors that have limited the progress in the development of such automated cleanup techniques are lack of module compatibility and lack of applications using flexible electronic controllers for automation of chromatographic procedures. We have undertaken the development of methods that can accommodate sequential chromatographic processes for fractionation of complex chemical residues. Contaminant enrichment and fractionation are achieved by sequential linkage of the described chromatographic modules. This approach was specifically developed for the enrichment of contaminants from aquatic organisms--particularly fish tissue (1).

We have measured the effectiveness of these chromatographic enrichment modules by determining a contaminant enrichment factor (CEF) (2). This factor is defined as the ratio of the weight of initial extracted sample to the weight of residual coextractives present after processing multiplied by the fractional recovery of the contaminant(s) under study. We defined this ratio as the contaminant enrichment factor (CEF) (2).

Normally, the percent recovery of specific contaminants is stressed in describing a cleanup procedure. However, the CEF associated with the procedure(s) involved in sample cleanup offers an additional means of evaluating the overall effectiveness of the methods. The larger the ratio the greater the matrix rejection. Use of both recovery data and CEF will give a better comparison of procedures for enrichment of complex environmental residues, enabling analysts to evaluate procedural alternatives more accurately.

To automate contaminant enrichment using our chromatographic modules, we contracted for the development and construction of a prototype programmable chromatographic separation system (3). This system consists of a solvent delivery subsystem, a sample storage subsystem, a low pressure chromatograph, a fractionation unit, and a controller.

The controller is a microcomputer interfaced to a chromatographic system through various solenoid-activated valves. The computer is programmed to closely control the chromatography and still provide the module selection to achieve the separations required. This flexibility is provided through independent control of the solvent and flow rate used for elution, introduction of sample, which column(s) used, the size and number of fractions collected, and the order in which the controller runs the chromatography steps.

MODULAR PROCEDURES

Gel Permeation Chromatography (GPC)

GPC is a form of liquid chromatography in which solute molecules - pollutants and biogenic materials - are selectively retarded as they permeate the pores in the column packing. Generally the larger nonaromatic lipid molecules are excluded from all or a portion of the pores by virtue of their size, and therefore elute from the column before the aromatic or smaller contaminant molecules. Long chain aliphatic hydrocarbons also have little interaction with these resins and are well separated from aromatic hydrocarbons. GPC gels have three advantages:

1. they can be used with a wide range of solvents,
2. they do not require complex solvent gradients, and
3. they can be used repetitively for non-polar compounds without chemical regeneration.

The technique, which has been automated (2), has been applied to the isolation of contaminants, such as pesticides, phenols, PAHs, and chlorinated aromatics--including chlorinated dibenzo-p-dioxins (CDDs) and dibenzofurans (CDFs)--from fatty acids, oil components, triglycerides, and other biological materials (2,3,4). The CEFs for GPC have ranged from 50 to 200 for lipid-rich extracts (2).

Cesium Silicate Chromatography

Simplification and automation of procedures for isolation of acidic pollutants such as phenols is critically needed for the analysis of large numbers of samples. Our modification of a method published by Ramiljak et al. (5) has circumvented many problems associated with isolation of acidic materials (6). Application of this procedure using columns of silica gel treated with cesium hydroxide (Cs-silicate) yielded significant improvements in the selective recovery of phenols from environmental samples. These columns, when placed in sequence with the GPC eluate (1:1, v/v, methylene chloride/cyclohexane) consistently retained the 11 priority pollutant phenols plus 2,4-dichloro- and 2,4,5-trichlorophenoxy acetic acids (4). A solvent mixture, 5 mL of 40% methanol in acetone, is sufficient to quantitatively recover all of the priority pollutant phenols. Neutral compounds such as PCBs, CDFs, and CDDs are not retained. The columns are regenerated after they are washed with 40 mL of the GPC solvent mixture.

Phenolics are determined as their anisoles or their pentfluorobenzyl ethers by electron capture GC or negative ion GC/MS. This procedure is currently being evaluated for its ability to separate acidic compounds in samples provided from our laboratory's monitoring programs for environmental contaminants in fish.

Dispersed-Carbon Chromatography

Our development of a method for dispersing finely divided carbon on the surface of shredded polyurethane foam (7) is an improvement over granular carbon columns which required large solvent volumes to elute strongly adsorbed contaminants. The carbon particles adhere strongly to the foam and are not dislodged during chromatography. The increased porosity of this support results in increased solvent flow, greatly facilitating the use of carbon as an adsorbent.

Although shredded urethane foam is an excellent support for carbon, the foam can be degraded by ultraviolet light. In an effort to overcome these problems, we examined several alternative supports for the dispersal of carbon.

Sample interferences are reduced by using carbon dispersed on glass fibers. Consequently, a large-capacity contaminant enrichment module based on carbon-glass fibers for isolating planar aromatic contaminants was developed. We also explored the combination of this module with other auxillary cleanup procedures that could be readily incorporated into a sequential configuration (2).

APPLICATIONS OF CONTAMINANT ENRICHMENT MODULES

Analysis of CDDs and CDFs in Aquatic Samples

Recently, we have applied our modular approach to the enrichment of CDFs and CDDs from extracts of aquatic organisms. CDFs are produced when PCBs are subjected to incomplete combustion as in open burning or pyrolysis, and CDDs are known contaminants in several widely used chemicals (8). Detection of CDFs and CDDs was sought because several of their isomers have been shown to be a highly toxic to mammals (9).

Demonstrating the occurrence of CDFs and CDDs in the aquatic environment has proved to be a formidable task for analysts because these contaminants occur in very low concentrations, and interfering components are present in fatty tissues (10). Highly effective cleanup techniques are required to enrich extracts containing these contaminants. Using the dispersed carbon module sequentially linked to the GPC module, we demonstrated that the CEF was approximately 10,000 for CDDs and CDFs (2). Use of these modules thus provided a means of isolating these compounds in quantities suitable for screening by negative ion mass spectrometry, by direct probe or GC/MS. The MS analyses for CDD and CDF residues in selected fish, turtle and seal fat samples were performed by Dougherty and coworkers (11).

In these analyses, CDFs having 4 to 8 chlorines were detected for the first time in aquatic environmental samples. The samples studied included relevant blanks, as well as fish samples from the Hudson, Ohio, and Connecticut rivers and Lake Michigan, containing PCBs in the range of 20-200 ppm.

In addition, fat of snapping turtles from the Hudson River and of Baltic gray seal from the Gulf of Bothnia were examined. The turtle fat was found potentially contaminated by penta- and hexachlorodibenzofurans and seal fat by pentachlorodibenzofurans. The concentration of PCBs exceeded 750 ppm in the turtle fat samples and was approximately 10 ppm in the seal fat. Chlorinated naphthalenes were also detected in all samples analyzed.

A more definitive analytical procedure was used because the direct probe high resolution MS technique employed is only a screening tool for CDFs. The method of choice was high resolution GC/MS which Buser and Rappe in several publications (12,13,14) have recently applied to the separation of CDD and CDF isomers at levels corresponding to part-per-trillion residue concentrations.

The snapping turtle and seal fat samples were further analyzed by Rappe et al. (14). Aliquots (2 ul) corresponding to 0.1 and 0.8 g of fat of the snapping turtle and seal, respectively, were injected into a 50 m glass capillary column (OV-17 or Silar 10C) coupled by way of a platinum capillary interface directly into the ion source of a Finnigan 4000 quadrapole GC/MS. The instrument was operated in the negative chemical ionization mode and methane was used as the reagent gas. Under these conditions the CDFs exhibit intense negative molecular ions (M^-) with limited fragmentation resulting from the addition of H and loss of Cl, to yield $(M-34)^-$ ions.

Complete spectra (m/e = 80-800) were recorded for the major GC peaks observed. Identification of a GC/MS peak as a CDF was based on the presence of the proper M^- ions and intensities of the ion clusters due to the chlorine isotopes. Identification of isomers was based on the retention times of authentic standards on both OV-17 and Silar 10C columns.

The total CDFs in the turtle fat sample was estimated at 3 ng/g. Of the individual CDF isomers, the toxic 2,3,7,8-tetra- and 2,3,4,7,8-pentachlordibenzofuran were estimated to be 0.045 and 0.62 ng/g, respectively. The levels of CDFs in the seal fat sample were much lower than in the turtle fat. In this sample the total CDFs were estimated as 0.04 ng/g, and the 2,3,7,8-tetra- and 2,3,4,7,8-pentachlorodibenzofuran as 0.001 and 0.015 ng/g, respectively.

ANALYSIS OF POLYCHLORINATED TERPENES (TOXAPHENE) IN FISH

Toxaphene is an insecticide that has been produced in very large amounts primarily for use in controlling cotton pests (15). Because it is a complex multicomponent mixture of over 150 polychlorinated terpenes, it has often escaped detection in aquatic residue surveys.

We have examined the occurrence and nature of polychlorinated terpene (PCTerp) residues in fish samples collected as part of the National Pesticide Monitoring Program. Residues derived from toxaphene have been frequently encountered in fish from the Southern United States, at levels as high as 20 - 100 ug/g (4).

Our awareness of PCTerps as widely dispersed contaminants in the aquatic environment began in 1976, when extracts from lake trout fry were referred to our laboratory by the Great Lakes Fishery Research Laboratory, Ann Arbor, Michigan, for examination by electron impact mass spectrometry. Toxaphene-like residues were found, yet it is believed that very little toxaphene had been used in the watershed. These data posed several questions as to the nature of the contaminants so detailed analysis was required.

Characterization of the residues in these samples was difficult because of the presence of many other contaminants (DDE, DDT, PCBs, etc.) which were not separated from PCTerp's. An improved sample cleanup scheme and means of detection was needed to eliminate the interferences to toxaphene-like contaminants. A cleanup procedure using nitric acid- sulfuric acid treatment of extracts was adapted to this problem and it proved suitable for removal of most compounds (including PCBs, though they are removed by silicic acid chromatography) interfering with the detection of PCTerps in fish extracts. However, most chlordane components were not removed and they are present as early eluting GC interferences to PCTerp analysis.

Screening of samples for PCTerps can be done with GC by using temperature programming and an electron capture detector. Although toxaphene or PCTerps can be detected by electron capture GC, their identity cannot be confirmed by this technique. Electron impact-MS is not a good means of confirmation for PCTerp residues in complex environmenal samples because their fragmentation patterns are complex and response factors are low. Before definitive analyses of the residues in environmental samples could be made by GC/MS, both the sensitivity and specificity of the GC/MS technique needed to be enhanced.

The complex fragmentation of PCTerps in electron impact mass spectrometry makes it difficult to obtain clearly defined molecular weight data. CI-MS had such poor response to PCTerps that it is not very useful in the analysis of environmental samples (16). Negative ionization mass spectrometry (NI-MS) with glass capillary chromatography columns, significantly improved our ability to characterize PCTerp residues.

The use of NI-MS, in contrast to EI-MS, yields both greater sensitivity and less molecular fragmentation (17). Thus NI-MS has distinct advantages over other techniques for analysis of toxaphene or other PCTerps in environmental samples.

This technique was applied to several sample extracts containing PCTerp residues. Residues in fish from Llano Grande Lake, Texas, Lake Michigan, and in fat from Baltic Sea seals were compared with each other and with toxaphene. NI-MS spectra of PCTerps exhibited enhanced sensitivity relative to chlordane components and revealed similarities between the PCTerp residues in all of these samples.

Environmental residues contained several isomers of the more highly chlorinated PCTerps, which were not detected in toxaphene. Further research on PCTerp residue analysis is directed toward obtaining a more comprehensive profile of the PCTerp residues which are present in aquatic ecosystems and obtaining some insight into the transport mechanisms that have led to their global dispersal.

SUMMARY

We have defined contaminant enrichment as concentration of trace environmental contaminants(s) concomitant with at least partial elimination of biogenic material or other interfering chemicals. A contaminant enrichment module, then is a process unit designed to provide an increase concentration of the contaminant(s) relative to the total material present. Ideally, these modules are designed to do this in an integrated fashon. Mechanization and/or automation of contaminant enrichment can readily result from the ability to sequentially link different chromatography modules.

Measurement of CEFs provides insight into the potential utility of individual and integrated cleanup procedures. Modules should be optimized for the isolation of specific contaminants or classes of chemicals from any other part of the residues that might interfer with the quantitation of particular trace organic residues in environmental samples.

We have developed several modules which provide an automated system for enrichment and fractionation of environmental contaminants found in aquatic ecosystems. We have have designed and evaluated three modules:

1. Automated gel permeation chromatography for removal of lipids and other biogenic compounds, and the consequent enrichment of a wide variety of contaminants

2. Cesium silicate chromatography, for the enrichment of acidic contaminants or the removal of acidic compounds before carbon chromatography is used

3. Carbon chromatography (Amoco PX-21 carbon) dispersed on shredded urethane foam or glass fibers, for the enrichment of planar aromatic contaminants such as chlorinated dibenzofurans, dibenzo-p-dioxins, and naphthalenes.

Application of improved contaminant enrichment processes to trace organic analysis of environmental samples has increased our ability to undertake difficult analyses for low levels of contaminants (e.g., CDFs, CDDs, and chlorinated napthalenes) and has provided new means for isolating acidic contaminants such as phenols and phenoxy acids. These modules have proved to be complementary to newly developed modes of mass spectrometric detection in which NI-GC/MS is used.

Through cooperation with other investigators we have extended our knowledge of the occurrence of polychlorinated terpenes, dibenzofurans and naphthalenes in fish and other aquatic organisms. We used a combination of these modules for the enrichment of chlorinated dibenzofurans in environmental samples that contained high concentrations of PCBs. Using NI-GC/MS, we detected numerous dibenzofuran isomers samples of fat from snapping turtles of the Hudson River and from seals of the Baltic Sea. The highly toxic 2,3,7,8-tetra-, and 2,3,4,7,8-pentachlorodibenzofurans were among the isomers identified. We have also applied negative ion GC/MS to the detection and characterization of polychlorinated terpenes in fish from the Great Lakes and rivers of the Southern United States.

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A MULTICHANNEL, REMOTE CONTROLLED, TEFLON AND GLASS POSITIVE DISPLACEMENT APPARATUS FOR COLLECTING TRACE ORGANICS FROM ENVIRONMENTAL SOURCES

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ABSTRACT

The recent, rapid interest in isolating and identifying trace organic compounds from environmental sources has focused largely on studies of adsorbents, extraction techniques, and chromatographic methods. As we have recently shown (4). the most critical factors in the sequence are the most difficult to control: the reproducible capture of compounds from a complex matrix and the subsequent recovery of compounds from the adsorbent. Further, the volume reproducibility and type of sampling-grab, flow, or time-proportional-can substantially affect the validity of the sample to represent the source (1).

We have designed an all glass and teflon multichannel positive displacement sampler which is driven by 50 mL syringes held in precision machined mounts. Up to four combinations of sources and collection methods, such as simultaneous sampling of a pre and post process wastewater with each stream being independently collected on e.g., XAD resin or carbon, can be applied. Internal standards can be precisely metered into the sample prior to the collection device, and the effluent from an adsorbent bed can be collected for independent anaylsis.

This apparatus has been used to collect samples of raw and finished drinking water, samples from the Illinois River, and from municipal and industrial effluents, including an untreated coal conversion process wastewater. Four radio-controlled units are being used in a nationwide EPA-funded study of the movement of organics through municipal treatment plants (8-10).

We will describe the design and construction of the apparatus and its use for the simultaneous sampling of trace organic compounds on XAD and granular activated carbon.

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INTRODUCTION

The ability to identify and quantify organic compounds present at trace levels in the environment, and the use of these data for assessing environmental impact, depends upon the least accurate or reproducible segment of the sampling and analysis chain. Although sorbents for concentrating organic residues from the aqueous matrix have been developed and analyses have improved, methods for collecting samples are primitive. Further, most of the developments have been empirical since the theoretical constraints on the sampling process and in the propagation of errors through the chain are only now beginning to be identified.

Sampling Theory

Schaeffer and Janardan (1) and Schaeffer et al. (2) have examined the nature and quality of information obtained from grab and composite samples. Thus, if the analyzed concentration of a single grab (or sub-) sample is X, then X will have a certain distribution (of concentrations) with a mean u and a variance σ^2.

$$(1)\quad E(X) = u,\ \mathrm{var}(X) = \sigma^2.$$

The information content, termed the invariance (3), of a single grab is given by the reciprocal of the variance,

$$(2)\quad I_x = 1/\sigma^2.$$

If $X_1, X_2, \ldots, X_n$ are the values of n subsamples, and $w_1, w_2, \ldots, w_n$ their proportions in the composite such that $\sum w_i = 1$, then the result of compositing n discrete (sub)samples is

$$(3)\quad Y = w_1 X_1 + w_2 X_2 + \ldots + w_n X_n.$$

From this it has been shown (1,2) that the mean and variance of the composite are

$$(4)\quad E(Y) = u,\ \mathrm{Var}(Y) = \sigma^2 (1 + n^2\sigma_w{}^2)/n,$$

where σ_w^2 is the variance of the compositing proportions w_i. Since the information content of the composite is $I_y = (\mathrm{var}(Y))^{-1}$, the relative information of a composite and grab sample is

$$(5) \quad I_y/I_x = n/(1 + n^2 \sigma_w^2).$$

When precisely equal subsample sizes form the composite so that $w_1 = w_2 = \ldots = w_n$, then $\sigma_w^2 = 0$ and the composite contains n times as much information as does the single grab. However, if composites are formed so that $w_i = w_j$, then $\sigma_w^2 > 0$ and the advantage of compositing is reduced. If $\sigma_w^2 > (n-1)/n^2$, the information content of a composite is less than that of a grab sample.

Since the compounds of interest are assumed to be present at low levels, it is unlikely that toxicologic data in the range of interest exists. Further, because the levels are low, the ability to capture, isolate, identify and quantitate the compounds present in a single sample is poor as shown by Janardan and Schaeffer (4) and Janardan et al. (5). (The problem is increased up to n-fold by taking a grab sample rather than a composite with $w_i = w_j$, i.e., $\sigma_w^2 = 0$).

Consideration of the objectives for monitoring the environmental levels of organic compounds, coupled with theoretical results, and backed by field experience described subsequently, shows that load and not concentration information is required.

With large resources load information can be obtained without compositing. Since, for example, the estimated analytical costs alone for the priority pollutants exceed $2000/sample (6), most sources will probably only be characterized by a single sample; this is best accomplished by a composite.

The the analyst ultimately reports the concentration, C, of the compounds in the sample.* Hence, the total flow, V, over the sampling period must be measured in order to estimate load. The estimated load $\hat{L}$ is computed as

$$(6) \quad \hat{L} = C\,V.$$

Typically, flow proportional composites are taken. However, such composites provide biased estimates of load (7). Thus, the concentration of a component in the composite is

$$(7) \quad \hat{C} = \sum w_i \, C_i / \sum w_i$$

where w_i is the proportion of the ith subsample, with concentration C_i, in the composite. The load is the sum of the subsample loads

$$(8) \quad \hat{L} = \sum L_i .$$

From Eqs. 6-8, the load obtained from a flow proportioned composite with subsamples of size $w_i = w_i$ is estimated from the measured concentration as

$$(9) \quad \hat{L} = \sum w_i{}' \; w_i c_i = \sum w_i{}^2 \, c_i .$$

Since the true load estimate $\hat{L}$ is a linear function of the subsample loads (Eq. 8), while the load estimated from the concentration of the flow proportioned composite is a non-linear function of subsample loads (Eq. 9), flow proportioning results in a biased load estimate. This is in addition to the loss of information due to compositing given by Eq. 5.

The conclusion from theory, which is supported by field studies, is that the type of information which will be most useful for assessing the environmental impact of trace levels of organic compounds is loading data obtained from constant volume (time proportioned) composite samples. Taken together, the theoretical results define the sampling requirements which consequently specify the operational requirements of the composite sampling device. The purpose of this paper is showing how the sampling and operational requirements are met by a new composite sampler.

SAMPLER DESIGN AND OPERATION

Design Requirements

The sampling system has to be field portable. Since samples will be taken over hours to days, it must be able to operate unattended. This requires that it be reasonably immune to suspended solids, that it be capable of operating at below freezing temperatures, or in hot weather with sample cooling. Further, since many of the compounds are chemically reactive or physically sorb on wettable surfaces, all surfaces in contact with the sample have to be relatively inert and non-wettable, such as teflon and glass.

No single concentration/recovery sequence works for all compounds (5). Also, it may be important to simultaneously characterize more than one source. These require equipment capable of taking equal volume samples using any combination of sources and collection methods, simultaneously.

A review of commercially available composite wastewater samplers revealed none ideally suited to our purposes. Consequently, we designed, built, and are using a multichannel sampler. It is light weight and rugged, easy to repair in the field, and it meets the other constraints and needs identified above.

Design and Operation

The sampler box and drive system are aluminum. The pump and plumbing are teflon and glass. The drive mechanism is shown in Figure 1. The cylinder piston combinations are standard 50 cc infusion syringes which are held tightly and accurately in machined mounts (Figure 1a). The plungers are removably fitted to the moving drive arm which is connected by a rod (Figure 1b) to a pinion (Figure 1c) on the drive cam (Figure 1d). Sample volume/cycle is determined by the position of the pinion on the precisely machined drive cam.

The syringes are connected via teflon lines to the common ports on the four channel hydraulically isolated two position valve shown in Figure 2. The body of the valve is machined teflon lined with a precision bore glass tube (Figure 2b) which has had ports diamond drilled through it. A teflon valve spool which is relieved to allow bi-directional flow and stiffened internally by a stainless steel control rod, slides inside the tube. The control rod is fitted with a spring return and is connected to a large solenoid (Figure 2a).

In typical operation, sample collection is triggered by a signal received by the electronics from an internal or external source. This latches a relay which places the drive cam in motion. As the drive cam turns through the first 180 degrees, sample is drawn through the intake port of the valve (Figure 2b) and into the syringes. A microswitch is then tripped energizing the solenoid which drives the valve spool to the sample expulsion position shown in Figure 2b. At this point (180-360°) the syringes are closing. Fluid which has collected in the syringes and in the lines between the syringes and the valves is forced to the outlet port of the valve. As the drive cam completes its 360 degree cycle, another microswitch is tripped which turns off the pump motor and de-energizes the solenoid which allows the valve to return to its original position. On a given cycle, 10, 20, 30, or 40 mL of sample can be passed through each channel of the system. Ten or more samples of a given size can be taken each minute. This pump can, therefore, deliver large volumes of sample in a short period of time.

Extremely accurate and reproducible sample volumes (i.e., $S_w^2 \sim 0$) can be delivered at pressures up to 50 psi, regardless of outlet head. This is particularly important when solid sorbent columns are used since the head loss across the columns can increase dramatically over the length of the run as suspended solids collect toward the inlet side of the columns. While differences in the column packing (e.g., open channel, carbon, etc.) mesh can cause different delivery pressures at each channel from the start of sampling, accumulation of suspended matter during the course of the run can cause widely differing outlet pressures on each channel of the valve. Delivery rates of peristaltic and bellows-type positive displacement pumps are highly dependent on outlet head. This results in a bias favoring the earlier subsamples. Piston type pumps, however, are largely immune to delivered volume variations due to outlet head.

Sampler electronics are located remotely in a control box. The motor and solenoid valve are controlled by electro-mechanical relays. Timing is accomplished through the use of microswitches located around the drive cam. Power is supplied through a simple regulated 12 volt supply.

The sampler must be able to sample both on a time and flow proportional basis. This is accomplished by using a high impedance relay driver which actuates the (relatively) high current relay coils using the small current available from the digital timer or from the dry contact read relays of commercially available flow meters. The interface, which is shown schematically in Figure 3, is capable of initiating a sampler cycle from a positive logic signal from transistor-transistor logic or from CMOS Logic at a signal level of approximately 0.5mA. Alternatively, an input is also provided for extremely low current signals from dry contact reed relays installed in most flow meters.

This high impedance input can be used for switching the sampler on when a particular liquid level is reached by using a simple probe-type level monitor consisting of two wires. The high impedance input of the interface requires a current of about 1 microamp to initiate sampling.

The present version of the timer is PMOS/CMOS quartz controlled type capable of timing in 16 ranges from 1 millionth of a second to 1 pulse every 464 days over 4 decades. It requires almost no power.

APPLICATIONS

Nationwide Study of Sewage Treatment Plants

A computer-based system of 4 samplers is being used by DeWalle and Chian et al. (8-10) to study the movement of priority pollutants through municipal wastewater treatment plants. Samplers are placed at strategic points in the plant: i.e., at the headworks, after the aeration basins, prechlorination, and post chlorination. The sampler control boxes are plugged into radio receivers. An ultrasonic flowmeter positioned near the headworks is plugged into the computer. The computer queries the operator on the flowmeter interval desired, and plant hydraulic data is entered through a standard teletypewriter keyboard/CRT. Upon keyboard command, sampling is initiated by the computer.

The computer follows a 24-hour slug of water through the plant in real time and samples it in a flow proportional manner. The firmware currently assumes plug flow, but any hydraulic model can be used. Recirculation can be accounted for, etc.

Coke Plant Sample

In our studies (11), the sampler has been used for collection of samples from industrial process effluents surface water, and POTW effluents. The most recent sample was a time proportioned composite of an untreated coking plant wastewater. Samples were simultaneously collected over 20 hours on macroreticular resins and in glass bottles. An internal standard solution of 2-ethylhexanoic acid and perdeuteroanthracene in absolute ethanol was continuously metered into the intake line of the sampler. The sampler performed well on the very hot and heavily silted sample. The data from this sample will be reported elsewhere (12).

CONCLUSION

Based on experience gained over several years of sampling for trace organics, and on the theoretical statistical aspects of the sampling process, we have designed and built a unique water sampler. It is capable of meeting theoretical requirements such as accurate sample volume, and the practical requirements of compactness, portability, and ease of service. Experience is now being gained with this equipment by us (5, 11-12), and with a computer controlled system by DeWalle and Chian et al. (8-10) in a nation-wide study.

This equipment brings the art of sampling up to the standards required by the subsequent chemical analysis.

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Glave, Robert Teece, Gerald Mack and James Johnston contributed to the testing of the original version. William Lawrence and Owen Ray did the glassblowing and machining. Glen Berry prepared the figures and Debbie Ray and Sheila Hinkley typed the manuscript.

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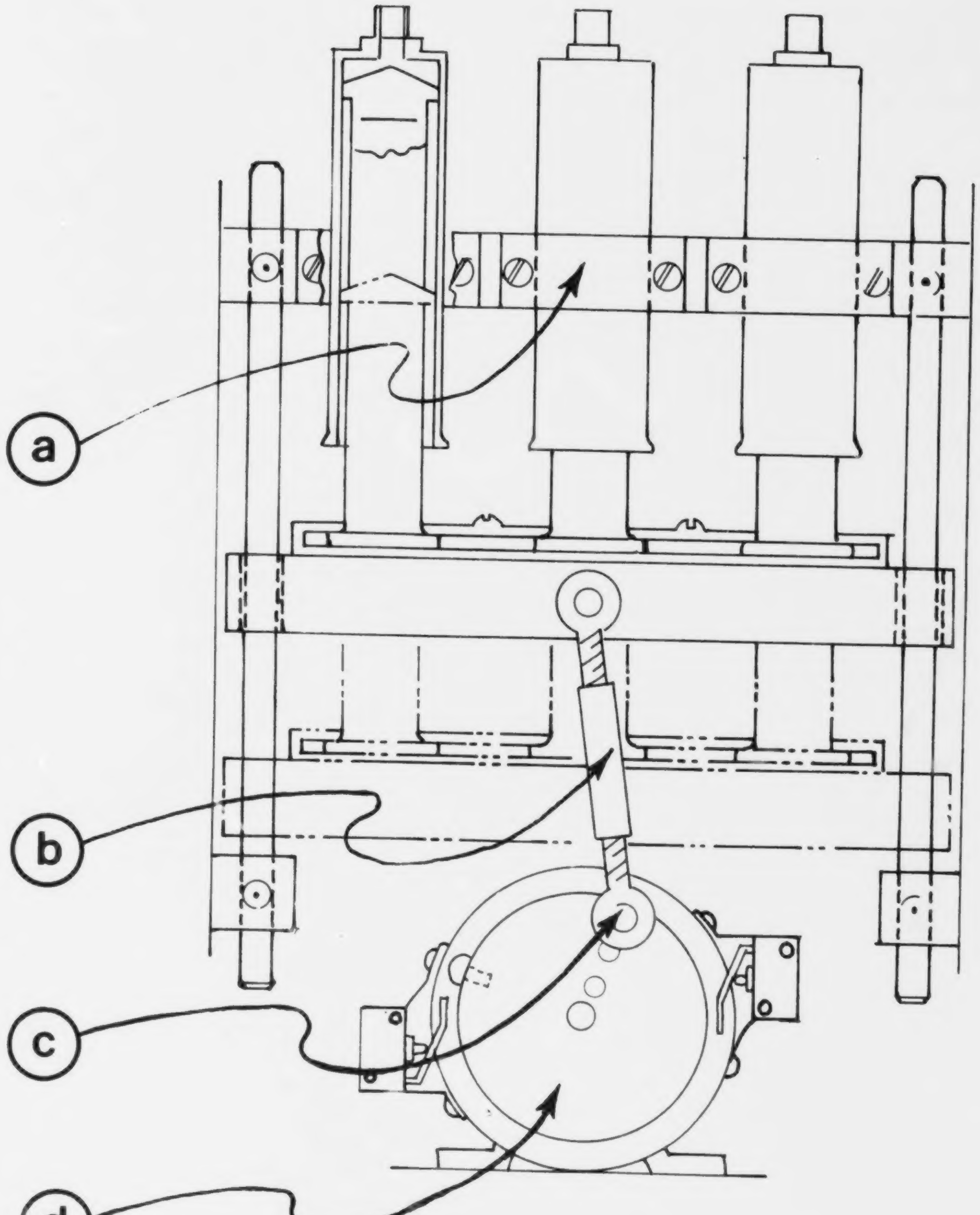

FIGURE 1 Mechanical components of sampler
(a is syringe mount, b is connecting drive, c is pinion, d is machined cam)

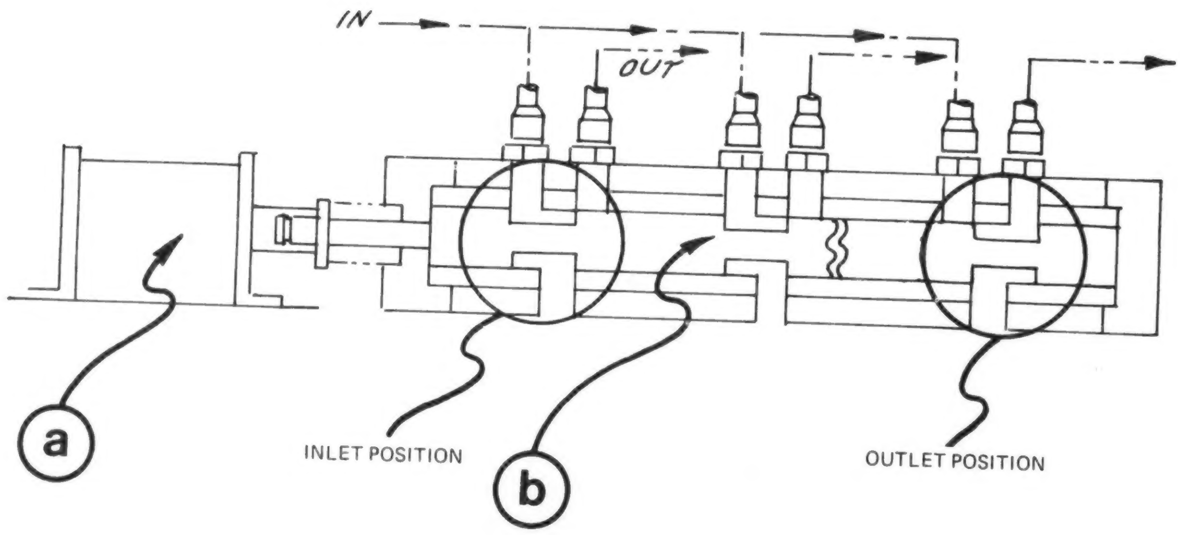
IN
OUT
a
INLET POSITION
b
OUTLET POSITION

FIGURE 2 Glass and teflon multichannel valve
(a is solenoid, b is precision bore glass tube with drilled ports)

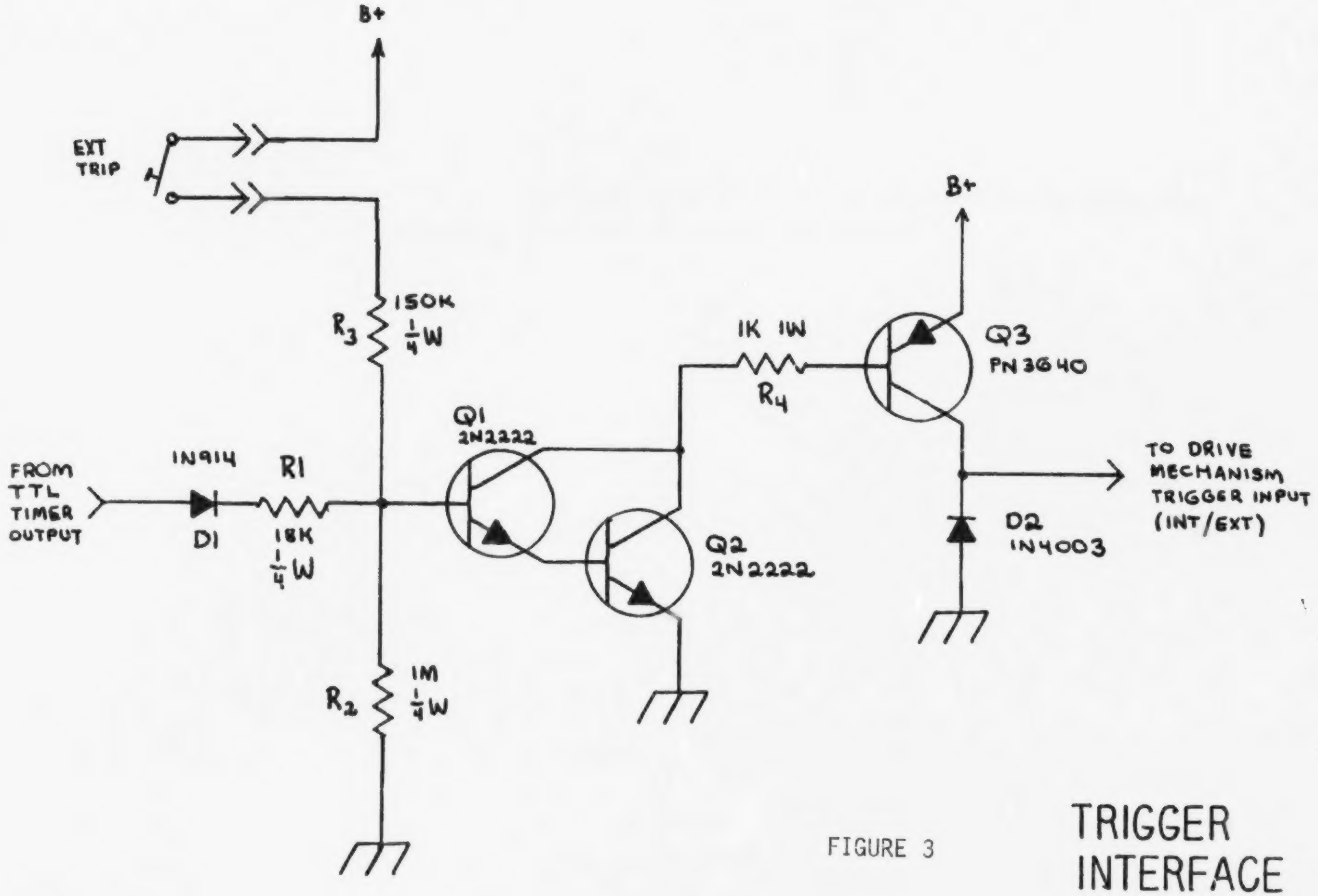
B+
EXT
TRIP
B+
150K
R_3
$\frac{1}{4}$W
IK IW
R_4
Q3
PN3640
Q1
2N2222
FROM
TTL
TIMER
OUTPUT
IN914
D1
R1
18K
$\frac{1}{4}$W
Q2
2N2222
TO DRIVE
MECHANISM
TRIGGER INPUT
(INT/EXT)
D2
IN4003
1M
R_2
$\frac{1}{4}$W

FIGURE 3

TRIGGER
INTERFACE
SCHEMATIC

PB82-211699

ION CHROMATOGRAPHIC ANALYSIS OF ORGANIC ACIDS IN DIESEL EXHAUST AND MINE AIR

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ABSTRACT

Low molecular weight carboxylic acids are among the highly water soluble compounds that are difficult to quantify by conventional organic analytical procedures. The relatively new technique of ion chromatography has the potential for analyzing these acids in complex matrices. Concentrations of two organic acids found in samples of diluted diesel exhaust and coal mine air were determined using two ion chromatographic procedures.

Samples were collected in midget bubblers containing dilute sodium carbonate solutions. Analysis of acetate, formate and fluoride ions in these solutions was performed using a 500 mm anion separator column and sodium borate eluent. Analysis of formate, acetate and acrylate in these solutions was performed using the ion chromatography exclusion mode (ICE) using the DIONEX IE-C-1 column with dilute hydrochloric acid eluent.

Retention time for formate ion using the borate system is 11 minutes at a flow rate of 2.3 mL/min. Fluoride, chloride, sulfate and acetate do not interfere in the analysis. Linear response is obtained over the concentration range of 0.1-4 μg/mL. The detection limit is estimated at 0.05 μg/mL for an injection volume of 100 μL at 3 μmhos full scale sensitivity.

The ICE system was used to confirm the identity of the acetate and formate detected in the samples and determine their concentration. The retention times of formic and acetic acids using the ICE system at an eluent flow rate of 0.7 mL/min are 16 and 23 minutes, respectively. No interference is observed from chloride, sulfate and acrylate. Linear response is obtained for formic acid and acetic acid in the concentration range of 1-20 μg/mL and 3-100 μg/mL, respectively. The detection limit for a 100 μL sample injection volume at 30 μmhos full scale sensitivity is estimated at 0.5 μg/mL. Concentration of samples by freeze-drying affords better detection limits with minimal loss of formic or acetic acid. Acrylic and carbonic acids are also analyzable under these conditions.

INTRODUCTION

Low molecular weight carboxylic acids may be present in emissions from combustion sources at part per million concentration levels (1). Formic acid and acetic acid both act as irritants to mucous membranes, eyes and skin (2). The emission goals established for the EPA-IERL environmental assessment programs are 5 ppm for formic acid and 10 ppm for acetic acid in gaseous effluents (3). Formic acid may also be produced in the environment by oxidation of formaldehyde emitted from combustion sources (4).

Previous attempts to measure concentrations of formic acid at ppm levels have been thwarted by inadequate detection limits. Generally, analytical methods for formic acid employ collection in aqueous solutions and reaction with oxidizing or reducing agents. Measurement of formic acid at high concentrations can be made by adding an excess of oxidizing agent and titrating the remaining excess of oxidant with reducing agents (5). Another method (6) relies on the distillation of a chloroform-formic acid azeotrope to separate formic acid from higher carboxylic acids and analysis of the formic acid by potentiometric titration with sodium hydroxide. Detection limits for this method are generally in the 0.1% range (6). Gas chromatography has been used to measure formic acid both directly and after derivitization. Direct analysis poses problems of corrosion of metal surfaces and of high detection limits with a flame ionization detector. Derivitization (7) eliminates these problems and achieves a detection limit of about 25 μg/mL.

Ion chromatography has recently been successfully used for analysis of formaldehyde after oxidation to formic acid (8) and thus can be used for direct analysis of formic acid.

In order to prevent interference by such inorganic anions as fluoride, chloride, and nitrate which occurs with $Na_2CO_3/NaHCO_3$ eluents, a weak aqueous eluent, i.e., $Na_2B_4O_7$, was used to achieve adequate separation. The detection limit in such ion chromatographic analysis is limited by the conductance of the suppressed eluents, but the development of ion chromatography exclusion (ICE), which permits separation of weak acids in a lower conductance background, has circumvented this problem. The determination of weak acids in complex media (9) has been reported with this technique.

This paper describes the analysis of formic acid and other carboxylic acids in diesel engine exhaust and mine air using ion chromatography (IC) and ion chromatography exclusion (ICE).

EXPERIMENTAL PROCEDURES

Collection

Organic acids in diluted (1:10) diesel exhaust or mine air were collected by drawing sample through two fritted bubblers (10) in series, each containing 15 mL of 10^{-3} $\underline{M}$ Na_2CO_3. A flow rate of 1.0 liter per minute and collection time of 60 minutes for diluted diesel exhaust or 240 minutes for mine air was used. A 37 mm glass fiber filter (Gelman Type A/E) was placed before the bubblers to remove particulates.

Analysis

The solution in each bubbler was transferred to a 25 mL volumetric flask and diluted to volume with the collection medium (10^{-3} $\underline{M}$ Na_2CO_3). In the case of samples collected from diluted diesel exhaust, an excess (3 mL) of this solution was flushed through a 100 μL sample loop of a Dionex Model 14 ion chromatograph. The sample was analyzed on the anion system and with instrumental conditions presented in Table 1.

TABLE 1

Ion Chromatographic Conditions

Instrument	Dionex Model 14 Ion Chromatograph
Eluent	0.005 M $Na_2B_4O_7$
Flow Rate	2.3 mL/min
Detector	Conductivity
Sensitivity	30 μmho/cm full scale
Anion Columns	3 x 125 mm Dionex Anion Pre-column 3 x 500 mm Dionex Separator (Borate Form) 6 x 250 mm Dionex Suppressor (H^+ Form)
Sample Volume	100 μL Sample Loop
Recorder	HP 7133A
Chart Speed	0.5 cm/min

In the case of samples collected from mine air, the bubbler solution was concentrated by freeze drying. Samples were transferred to wide mouth jars, frozen to -25°C and freeze-dried under 0.5 cm Hg vacuum in a Vacudyne, Inc. Pilot Freeze Dryer. Once reduced to dryness, 1 mL of distilled/deionized water was added to the samples. After shaking to ensure complete dissolution, 300 μL aliquots were placed in micro vials available for use in a Waters Associates Autoinjector Model 710 A. One hundred μL sample volumes were automatically injected into the ion chromatograph and analyzed on the ion chromatography exclusion (ICE) system and with instrumental conditions presented in Table 2.

TABLE 2

Ion Chromatographic Exclusion (ICE) Conditions

Instrument	Dionex Model 14 Ion Chromatograph
Eluent	0.0001 M HCl
Flow Rate	0.7 mL/min
Detector	Conductivity
Sensitivity	3 μmho/cm full scale
ICE Columns	9 x 250 mm Dionex Exclusion 3 x 500 mm Dionex Halide Suppressor (Ag$^+$ Form)
Sample Volume	100 μL, Waters Associates Autoinjector (Model 710 A)
Recorder	HP 7133A
Chart Speed	0.5 cm/min

RESULTS AND DISCUSSION

Analytical Method

Initial experiments were conducted on the Dionex Model 14 ion chromatograph to determine the feasibility and sensitivity of ion chromatography for analysis of formic acid. The conditions of IC analysis (Table 1) chosen were based on the need for separation of formate ion from the fluoride, chloride and acetate anions and for obtaining a suitable detection limit. Thus, the weak borate eluent (0.005 M $Na_2B_4O_7$) combined with a 500 mm anion separator column (and a 150 mm pre-column) were chosen. Conversion of the pre-column and separator column to their borate forms (from the normal carbonate form) was necessary, and the process of continuously passing the new eluent through the columns until a stable baseline was obtained required several hours.

Standard stock solutions of formate and acetate were prepared from reagent grade sodium salts. Mixed stock solutions were also prepared. Injections of 3 mL were made tc fill a sample loop of 100 μL volume.

A typical ion chromatogram of formic and acetic acid is shown in Figure 1. The retention times for acetic and formic acids under these conditions are 6.6 and 8.8 minutes, respectively. The preceding negative peak

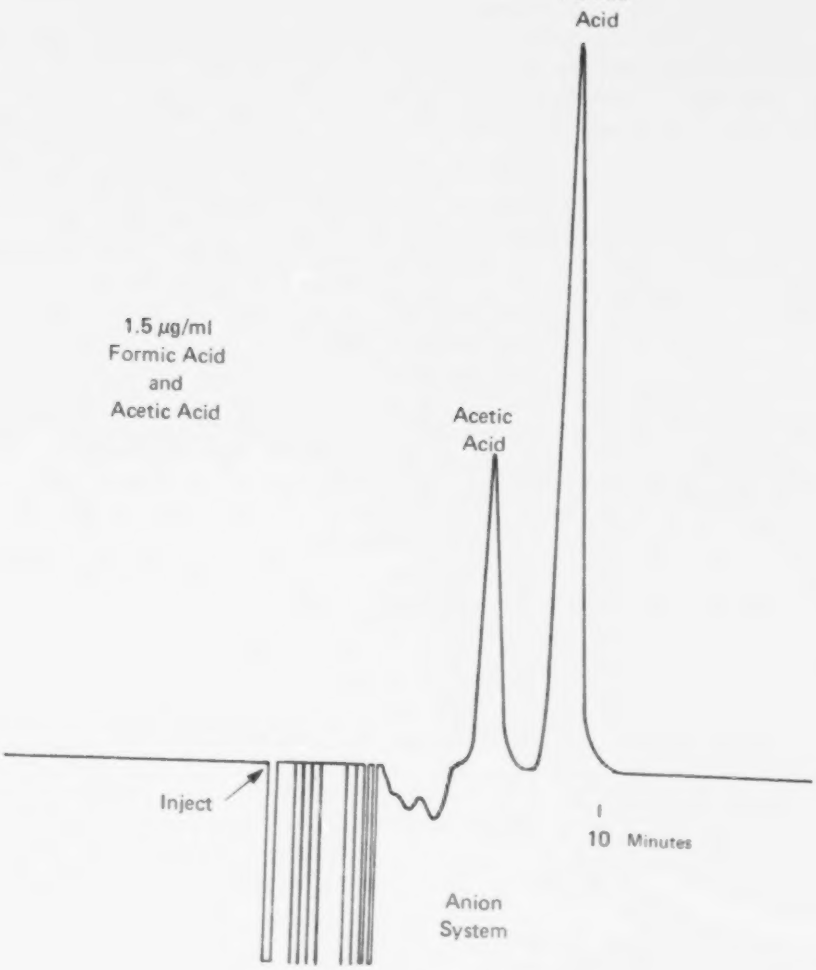


FIGURE 1 ION CHROMATOGRAM OF FORMIC AND ACETIC ACID

grouping seen in the 2-5 minute region is due to water whose conductivity is lower than boric acid. The peak height is linear with formic acid concentration over the range from 0.1 to 35 μg/mL. The corresponding linear range for acetic acid is about 0.2 to 70 μg/mL. The presence of fluoride or chloride in the sample does not interfere with the formic acid analysis. Retention time for these ions under the stated conditions are F^- (5.4 minutes) and Cl^- (29 minutes). Sulfate ion is retained for a longer time. Ultimately, these anions are eluted and may interfere with subsequent samples. Generally, these highly retained anions show very broad peaks, which are easily distinguished from the organic acid peaks.

Due to this interference, the need for frequent regeneration of the suppressor column and the ability of ion chromatography exclusion to easily separate organic acids and Cl^- and $SO_4^=$, an ICE system was tested for similar analyses. Typical ICE conditions (Table 2) provide easy separation of strongly ionized species (e.g., H_2SO_4, HCl, HNO_3) from weakly ionized species (e.g., organic acids) due to the greater retention of uncharged species in the interstitial fluid of the packing material (9). The weakly acidic nature of the eluent influences the ionization of each organic acid in proportion to its pK and thus its retention time. A 10^{-4} $\underline{M}$ hydrochloric acid eluent offers adequate separation of the strong acids and the organic acids of interest with a reasonable retention period. Standard stock solutions were prepared as before and injected onto the ion chromatograph. In order to utilize very small sample volumes, the normal sample loop, which requires excess sample, was by-passed and a Waters Associates Autoinjector (Model 710 A) installed. One hundred μL injections were made directly into the flowing eluent by the autoinjector and analyzed on the ICE system. A typical ion chromatogram of formic acid and sulfuric acid is shown in Figure 2. The retention time for the sulfuric acid and formic acid are 9.8 and 15.4 minutes, respectively. The peak height is linear with formic acid concentration over the range of 0.3 to 10 μg/mL. The corresponding linear range for acetic acid is 3 to 100 μg/mL (Figure 3). As well as providing separation of these organic acids and precluding interference from strong acids, the ICE system permits continuous analysis of samples for up to 30 hours without regeneration of the suppressor column.

The precision of the ICE method was determined by analyzing six replicates of two standard solutions containing strong acids (i.e., H_2SO_4) and both formic acid, acetic acid and carbonic acid. Carbonic acid is present as a result of the use of Na_2CO_3 in the standard solution matrix (as in the collection medium) and dissolution of atmospheric carbon dioxide (Figure 4). At formic acid concentrations of 5.0 and 10 μg/mL, the measured mean concentrations (Table 3) were 5.08 and 10.0 μg/mL, respectively. The relative standard deviation (CV) was 0.025 and 0.016, respectively.

Collection Method

The goal of the initial phase of our work was to determine the concentration of formic acid in engine exhaust subject to different forms of control, e.g., catalytic oxidation. Initially, samples were collected from diesel engine exhaust diluted by a factor of 10 in a stainless

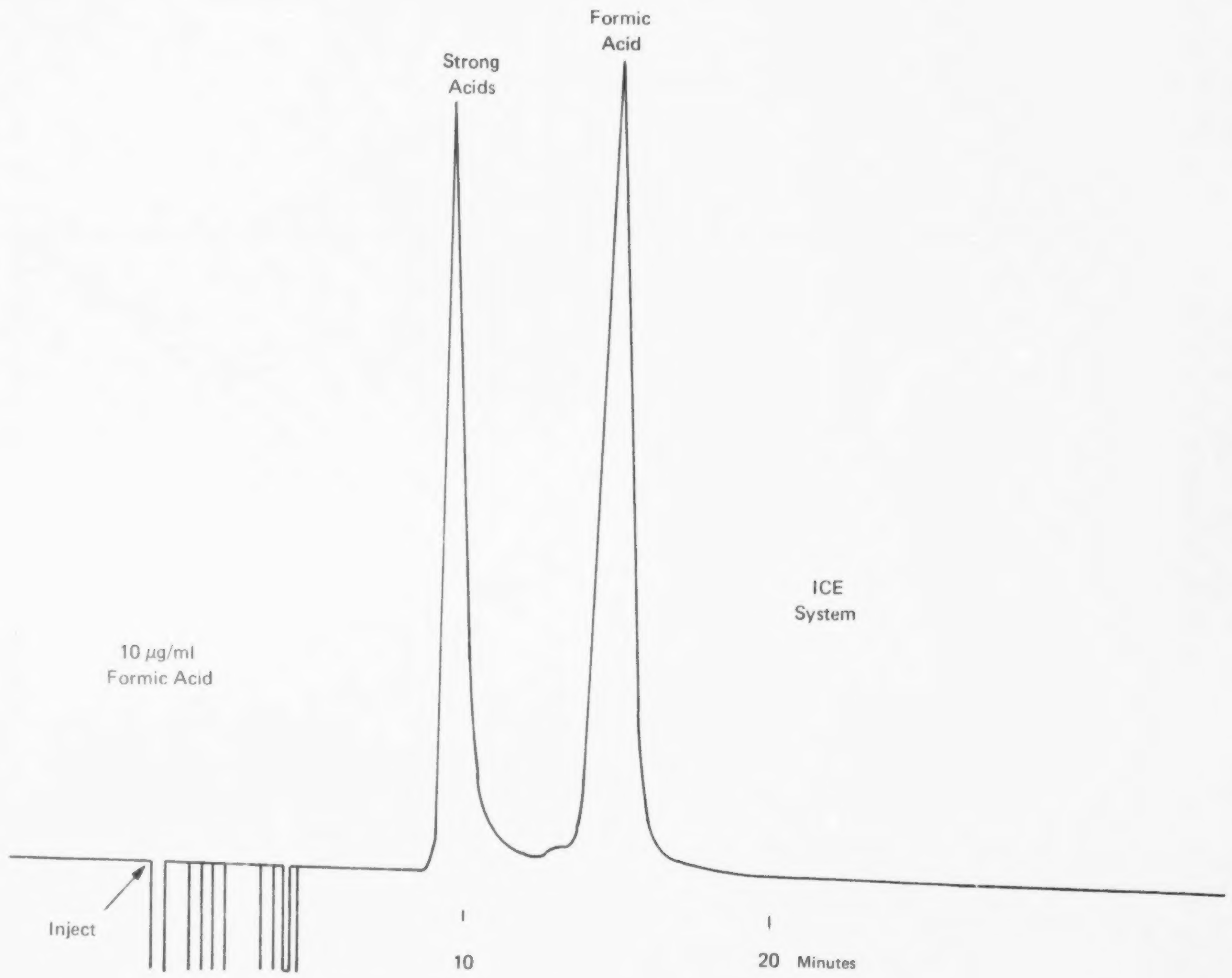
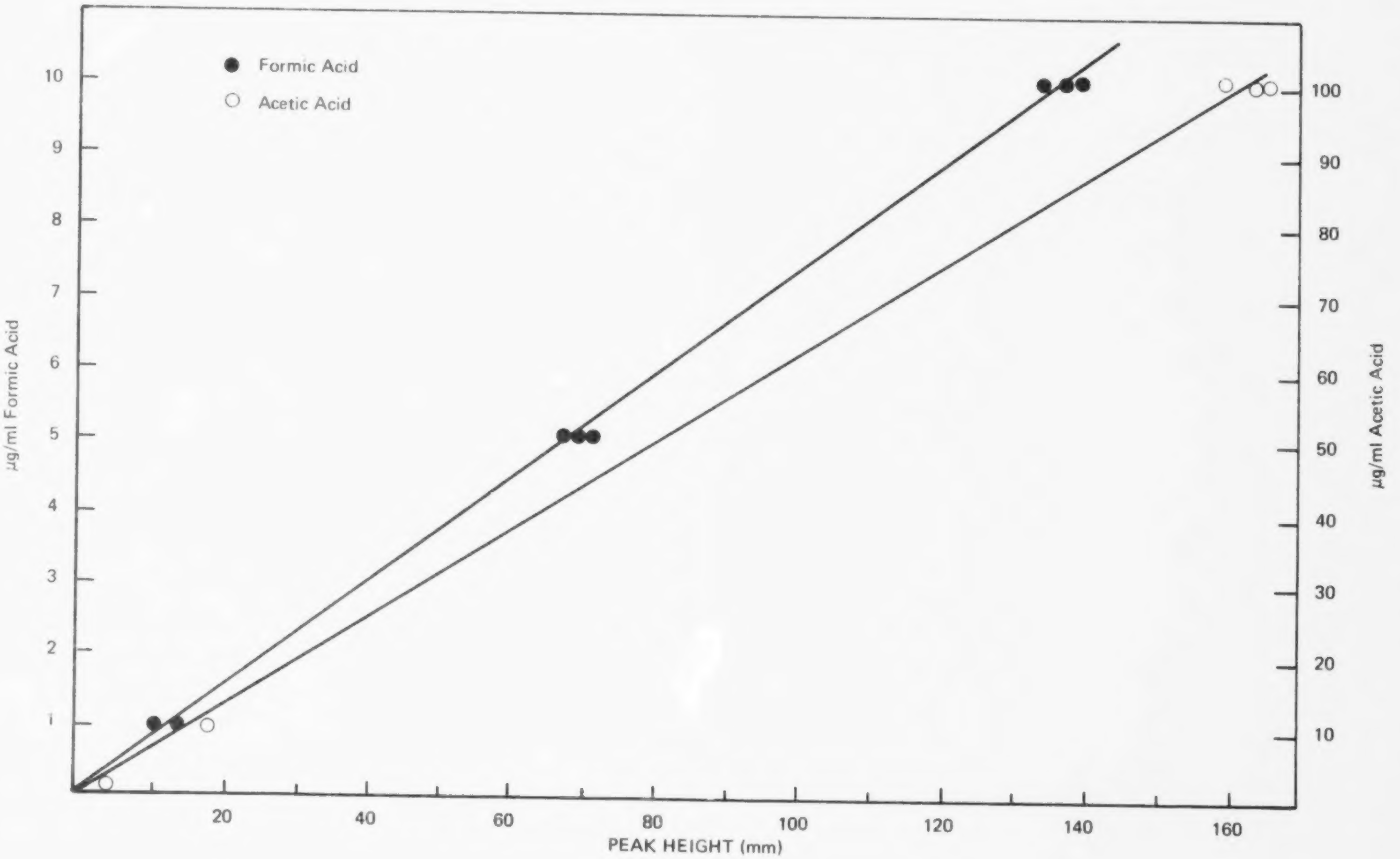


FIGURE 2 ICE CHROMATOGRAM OF FORMIC ACID AND OTHER STRONG ACIDS



STANDARD CURVE

FIGURE 3 ICE SYSTEM

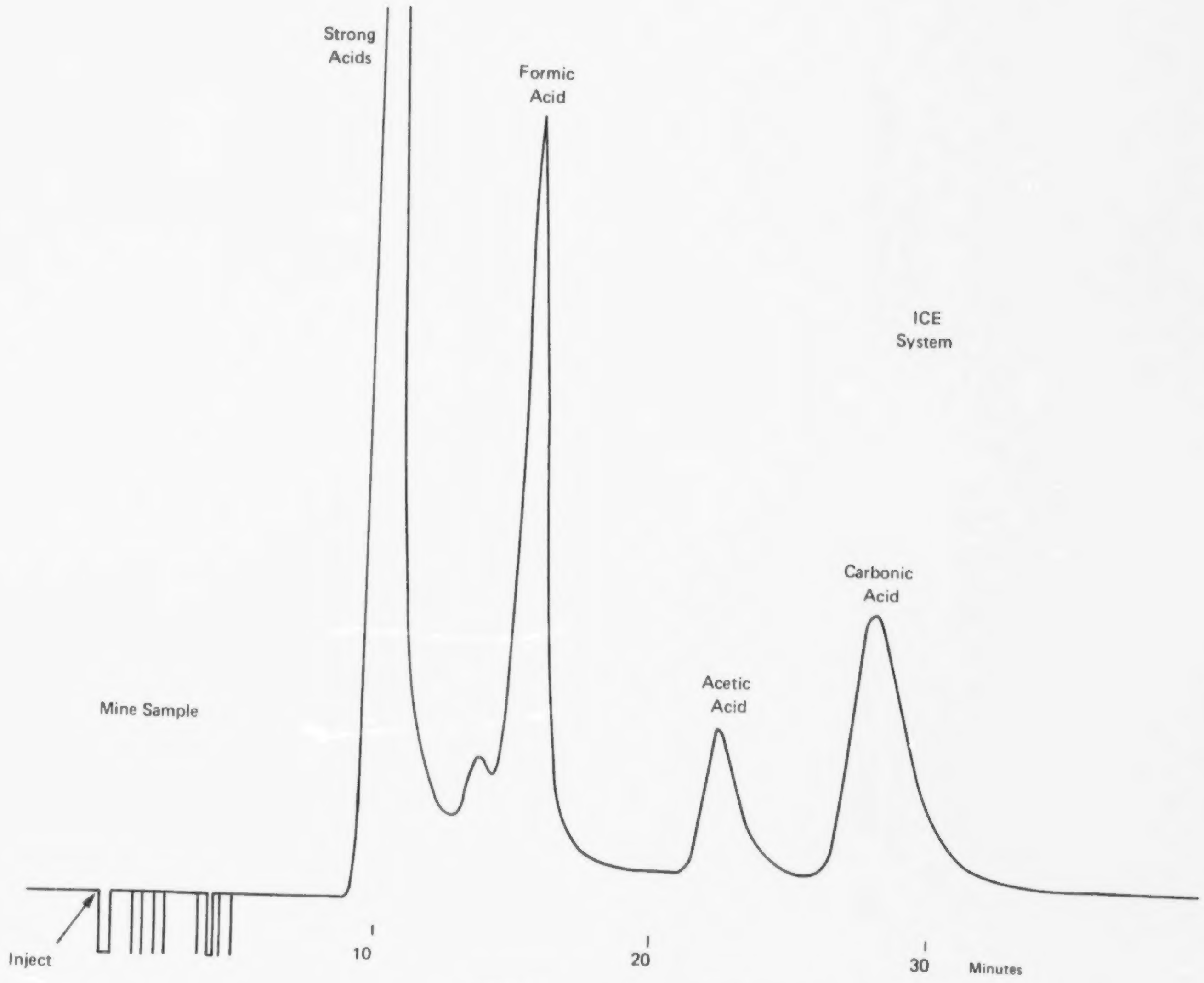


FIGURE 4 CHROMATOGRAM OF MINE SAMPLE

TABLE 3

Analytical Precision

Formic Acid

Calculated Concentration (μg/mL)		Observed Concentration (μg/mL)
5.00		5.23
		5.18
		5.18
		4.95
		4.95
		5.01
	Mean	5.08
	Std Dev	0.127
	CV	0.025
10.00		10.2
		10.2
		10.1
		9.9
		10.1
		9.8
	Mean	10.0
	Std Dev	0.163
	CV	0.016

steel dilution tunnel. A collection medium of 15 mL of 10^{-3} $\underline{M}$ sodium carbonate solution was utilized for two reasons. First, such an aqueous solution (7) is reported to provide good collection efficiency of soluble organic acids at flow rates of 0.5 to 5 L/min. Second, due to the volatility of free formic acid, it was felt that a basic solution would provide improved stability of the samples over periods up to seven days. The collection efficiency of this medium was determined by measuring the amount of formic acid collected at 1.0 L/min in two bubblers connected in series. The amoung of formic acid found in the front and back bubbler at three challenge concentrations are reported in Table 4. The data show that the collection efficiency is greater than 92%.

TABLE 4

Collection Efficiency

Challenge Concentration (mg/m^3)	µg Formic Acid Found Front	Back	% Collection in Front
12	153	<2.5	>98.4
6.7	90.0	<1.0	>98.9
2.5	32.5	<2.5	>92.3
0.06	10.3	0.8	92.2

In order to provide at least 99% collection of formic acid, two bubblers were routinely used in series. In a later phase of work, the formic acid concentration in mine air subject to diesel emissions was measured. The expected concentrations were about one hundred times lower than those found in engine exhaust. The efficiency of the collection scheme was again measured under these conditions of challenge concentration (0.06 mg/m^3). The collection efficiency was found to be 92.2% at this level (Table 4).

Sample Analysis

For sample collection in both diluted diesel exhaust and a mine atmosphere, the collection technique described previously was used, but the sampling periods were 60 minutes and 240 minutes, respectively. In the mine samples, the amount of formic acid collected was too small to be analyzed reliably after dilution to 25 mL. This problem could be precluded by collection at a higher flow rate (e.g., 5 L/min) or longer time periods. However, in order to utilize the samples as collected, they were concentrated by freeze-drying. The pH of samples prior to freeze-drying was checked to ensure that solutions were slightly basic. Strongly acidic species, e.g., H_2SO_4, collected during mine air sampling, slowly deplete the Na_2CO_3 in the collection media and weakly ionized organic acids may be volatiled at low pH. Recovery of formic acid during the freeze-drying process was checked by carrying standard solutions through the entire analytical procedure. As is shown in Table 5, recovery of triplicate samples of two concentrations averaged about 0.88.

TABLE 5

Formic Acid
Recovery After Freeze-Drying

Initial Sample Concentration (μg/mL)	Freeze-Dried Sample Concentration (μg/mL)	Recovery
4.3	3.9	0.91
	3.7	0.86
	3.9	0.91
9.7	9.0	0.93
	8.3	0.86
	7.8	0.80

Storage stability of these samples was checked by replicate analysis after a period of seven days. Losses of less than 2% were observed after storage for this period of time.

The accuracy of the ICE method was assessed by using the standard addition technique. Four mine samples containing measured concentrations of formic acid of about 0.8 to 1.1 μg/mL were spiked with a known volume of formic acid standard solution sufficient to double the sample concentration. The observed concentration indicated agreement of better than ±12% with an average agreement within ±5% (Table 6).

TABLE 6

Standard Addition of Formic Acid

Initial Concentration (μg/mL)	With Spike Calculated	With Spike Observed	Agreement
0.8	1.6	1.8	1.12
1.0	2.0	2.1	1.05
1.1	2.2	2.3	1.04
1.1	2.2	2.2	1.00

Standard addition was also used to confirm the identification of the chromatographic peaks based on comparison of retention time. No interference was observed due to chloride, sulfate or carbonate ion. Both formate and acetate were adequately resolved. A small unidentified peak did precede formic acid but did not significantly affect quantitation.

CONCLUSIONS

Two ion chromatographic techniques were developed to quantify formic and acetic acid in both diesel engine exhaust and mine air subjected to diesel emissions. A commonly reported anion separation system utilizing a weak borate eluent adequately separated the acids of interest in diesel exhaust. It was, however, affected by the presence of strong acids during subsequent consecutive analyses.

In order to preclude this problem and the necessary frequent regeneration of the anion system's suppressor column, an ion chromatography exclusion scheme was utilized. Samples collected in a mine environment were reliably concentrated by freeze-drying and then analyzed on an ICE system with dilute hydrochloric acid eluent. The precision of the ICE method was experimentally determined to be ±2.5%. The accuracy was not independently determined but good precision and recovery yield confidence that measured values are within ±5% of the true value. No interferences were observed in the ICE system due to strong acids, carbonic acid or other water soluble species present in mine air subject to diesel emissions.

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A NEW ELECTROCHEMICAL APPROACH TO TRACE LEVEL ALDEHYDE AND KETONE ANALYSIS

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ABSTRACT

A new electroanalytical approach using chemically modified electrodes for the determination of specific classes of organic compounds is described and tentatively evaluated. The suggested method consists of two principal steps: the pre-concentration of the analyte at the electrode surface via a selective chemical reaction with the modifying molecule and the quantitation of the subsequent surface-bound reaction product via differential pulse voltammetry. By judicious choice of the chemically modified electrode and the pre-concentration reaction employed, the approach in principle may be applicable for the selective monitoring of several important families of organic compounds. In this work, however, a model analytical system applicable specifically for the determination of dissolved aldehydes and ketones is considered. The chemically modified electrodes employed for the analysis were formed by the adsorption of allylamine onto the surface of a platinum wire. The pre-concentration reaction involved the formation of an imine by condensing the carbonyl analyte with the attached amine. For the model analyte ferrocene carboxaldehyde, a detection limit of $10^{-7}\underline{M}$ was observed using a pre-concentration time of only five minutes. No major interference was observed when the analysis was carried out in the presence of a hundredfold excess of ferrocene.

INTRODUCTION

Environmental analysis systems are often called upon to perform quantitative measurements on chemical species present in only minute amounts in complex and sometimes highly interfering matrices. In many cases, the analyst's task could be compared to the job of locating the needle in the proverbial haystack. Furthermore, as the identity of the target analyte is sometimes not known until the results of the analysis are completed, the analyst is not even sure beforehand that a needle is what he should be looking for. As a result, the use of any analytical technique for the purpose of making environmental measurements generally imposes severe demands on the performance level required of the technique.

Among the most important criteria that must be considered in evaluating any environmental analysis approach are both its sensitivity and selectivity. Thus, because of high degree of specificity inherent in their separation step, chromatography-based methods have ranked among the most widely used for trace organic pollutant monitoring. Similarly, although most electroanalytical methods possess adequate sensitivity for such use, these methods are generally not specific enough to be used alone and have had their primary application as detectors at the end of gas or liquid chromatographs.

In the instances in which they are applicable, however, electroanalytical methods do present several significant benefits. By and large, the instrumentation involved in electrochemical techniques is comparatively inexpensive and simple to operate; and, even though they are not generally capable of ultra-trace level determinations, they routinely operate in the ppm range for electroactive species. In addition, the technique of anodic stripping voltammetry (ASV) offers a specific opportunity for orders of magnitude sensitivity enhancement by including a pre-concentration step in which the intended analyte is electrodeposited at the electrode surface prior to analysis. Unfortunately, ASV is mainly useful only for the analysis of metal ions since, unlike metals, most organics cannot be plated out and effectively pre-concentrated.

In this report, we will describe a new pre-concentration approach to the electrochemical analysis of organics. Our objectives are to add a dimension of chemical selectivity to the electrochemical approach while, at the same time, maintaining or increasing its usual sensitivity. The suggested method proposes the use of recently developed chemically modified electrodes (CME's) first to pre-concentrate a specific organic analyte at the electrode surface via a selective chemical reaction and then to quantitate the subsequent surface-bound reaction product via conventional voltammetric techniques. CME's are essentially ordinary electrodes whose surfaces have been altered chemically by the attachment of molecules containing particular functional groups. As a result, CME's possess a dual set of properties-electrochemical ones which are determined by the externally applied potential and chemical ones which are determined by the nature and reactivity of the attached molecule. In this work, the variable chemical properties of CME's will be utilized to allow the selective pre-concentration of organic analytes at the CME surface. In particular, the chemical reactivity of the surface-modifying functional groups toward specific classes of organics will be examined with respect to the sensitivity enhancement and the gross chemical selectivity that can be imparted by means of the pre-concentration step.

The experimental system which is considered below is applicable in principle for the determination of carbonyl-containing compounds. We will specifically employ ferrocene carboxaldehyde as a model analyte. This choice was made because ferrocene carboxaldehyde can react chemically (i.e., undergo pre-concentration) as an aldehyde and at the same time can easily be detected at the electrode surface by means of its distinctive and readily accessible oxidation to the ferricinium species.

BACKGROUND

In recent years several groups have been actively engaged in research directly involving the design and characterization of CME's (1-19,21-27) Both normal conducting (Pt, C, etc.) and semiconductor (SnO_2, TiO_2, and RuO_2, etc.) electrode surfaces have been altered by the attachment of numerous organic molecules yielding electrodes possessing a variety of interesting chemical properties.

In most cases, one of two general attachment approaches has been employed: (1) the covalent bonding of the modifying molecule to functional groups present on the native electrode surface or (2) the irreversible chemisorption of the modifier onto the electrode material. The former method, employing amide, ether, and silane linkages, has been shown to be quite general in scope, yielding surfaces that are permanently and reproducibly altered. The drawback of the covalent approach is that the chemical reaction employed in the binding step is usually non-trivial in nature, requiring special or severe reaction conditions and/or long reaction times (several hours to several days). The second approach, utilizing the direct adsorption of a variety of species (e.g., n-alkenes, polymers, etc.), also appears to be very general both in terms of the types of electrode meterials (Pt, C, etc.) that can be modified and the types of functionalities that can be attached. The adsorption itself is usually relatively permanent and can normally be accomplished by simply immersing the "clean" electrode in a dilute solution of the surfactant for a few minutes.

A listing could be given that encompasses each molecule and functional group that has been thusfar either bound or adsorbed onto an electrode surface, but it would be simpler for our purposes to summarize the compilation by making one very general observation from it. The reactivity-both chemical and electrochemical-of species attached to electrode surfaces remains roughly comparable to that of the analogous species present in bulk solution. Chemical reactions characteristic of the attached molecule have been demonstrated to occur at CME surfaces under relatively severe reaction conditions and without noticeably affecting the attachment itself. Examples include metal complexations, ligand substitutions, protonations, amine-aldehyde condensation, amidizations, etc. Similarly except for a few sterically prohibited situations (15), species that are electroactive in dissolved form have been shown to retain approximately identical redox behavior when present in surface-bound form (19). Thus, the response of CME systems possesses two different components, a chemical component determined by the nature and reactivity of the specific attached molecule and an electrochemical component determined by the specific potential at which the electrode is maintained instrumentally.

The main thrust of the work to date has been directed toward the development of generalized modification strategies and the verification of these modification procedures either by surface analysis of the resulting electrodes or by electrochemical characterization of the redox behavior of the attached species. Furthermore, in the application areas that have thusfar been reported for CME's, the primary goals that have been considered have been the formation of an electrode capable of participating in selective (9,10), catalytic (7,13) , or photo-assisted (22,27) Faradaic processes. But in only a few cases have the special properties of CME systems been exploited for the purpose of obtaining an electrode system of enhanced analytical ability. Lane and Hubbard (17, 18) have employed a platinum electrode coated with an adsorbed layer of iodide ion to prevent the electrode fouling and high background currents that normally occur in the in vivo analysis of dopamine and norephinephrine. And, in work more directly related to our own, Cheek and Nelson (4) have utilized a carbon paste electrode containing attached EDTA functionalities to pre-concentrate via chelation and subsequently to analyze via cyclic voltammetry silver ion present at concentrations as low as 10^{-11}M.

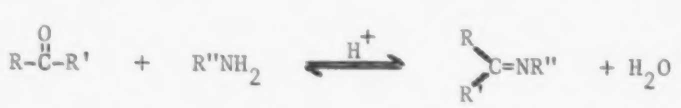
GENERAL PLAN OF WORK

The general requirements for an acceptable CME/pre-concentration system are the following:

1. The analyte involved should be one that is of direct analytical importance.
2. The CME involved should be one that can be formed rapidly and reproducibly.
3. The pre-concentration reaction should be rapid and straightforward, uncomplicated by side products and as specific as possible for the analyte of interest.
4. The reaction product should remain bound to the electrode surface and should possess a characteristic electrochemical activity.

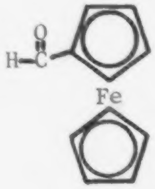
Finding organics which are of analytical importance is of course a simple matter. Similarly, because of the wide variety of CME's and modification methods that have been developed, procedures for the attachment of many common functional groups are already available; and, when the attachment is made via one of the adsorption approaches, the procedure required is elementary to perform. The most difficult aspect of the proposed approach appears to be the proper selection of the pre-concentration reaction. Among the sources that could be consulted in this regard is the literature of qualitative organic analysis which consists, in large part, of a compilation of straightforward and relatively specific chemical reactions suitable for functional group and family analysis. Further, many of the derivitization reactions developed for use in chromatography, mass spectroscopy, and optical analysis are also appropriate candidates for the pre-concentration step. Many of these reactions involve products that are expected to be electroactive and therefore should be suitable for the subsequent electroanalysis step.

A chemical system which meets several of these criteria and was consequently chosen as a model system for the work described here was one which is applicable for the analysis of dissolved aldehydes and ketones (25). The pre-concentration step utilizes the familiar acid-catalyzed condensation reaction between carbonyl-containing compounds and primary amines to give imine-containing products:



$$R{-}\overset{\overset{O}{\|}}{C}{-}R' + R''NH_2 \xrightleftharpoons{H^+} {}^{R}_{R'}{>}C{=}NR'' + H_2O$$

This reaction has been employed in the trace electroanalytical determination of dissolved amines (20,26) and has previously been shown to take place at amine-modified CME's (26). Since the imine bond formed as a result of this reaction is reducible at a readily accessible negative potential, it would in principle be feasible to quantitate the reaction product directly for any reactive aldehyde or ketone. For this study, however, ferrocene carboxaldehyde



was utilized as a model aldehyde analyte; and the characteristic oxidation of the ferrocene to ferricinium ion was employed for product detection and estimation. The technique of differential pulse voltammetry was used for electrode surface characterization and analysis purposes. Our objectives were to evaluate, first, the selectivity with which organic molecules containing a specific functional group can be concentrated at an appropriate CME surface and, second, the sensitivity with which such procedures can be used for quantitative analysis of the surface-bound species.

PROCEDURE

In this study, CME's were constructed from platinum wire, one end of which was sealed into glass tubing. Rigorous cleaning and pre-treatment of the platinum surface was essential for obtaining reproducible results. Prior to each experiment, the electrodes were heated to incandescence in a methane-oxygen flame and quenched in perchloric acid. They were then placed in an aqueous 0.1M $LiClO_4$ solution, and the potential was scanned slowly between +1.5 volts and -0.6 volts for several cycles and then held at +0.40 volts for at least three minutes. Figure 1A shows the typical current-voltage behavior observed following this pre-treatment regimen. This and all subsequent analyses of the coated and reacted electrodes were performed in 0.1M $LiClO_4$/acetonitrile using differential pulse voltammetry (potential scan rate: 2 mv/sec; initial potential: 0.0 volts; pulse amplitude: 50 mv).

Electrode modification was performed by adsorbing allylamine onto the clean platinum surface. This was accomplished by immersing the clean electrodes in a 0.5% allylamine solution for three minutes. A differential pulse voltammogram of the freshly modified CME is shown in Figure 1B and is seen to contain little dramatically different behavior from the clean electrode surface. Ferrocene carboxaldehyde pre-concentration was accomplished by immersing the allylamine-coated electrode in an acidified ethanolic analyte solution that was heated to 75°C. The differential pulse voltammogram of such a reacted electrode is illustrated in Figure 1C and shows the presence of a reversible redox couple at approximately +0.32 volts. The observed peak potential closely matches that found for the imine condensation product formed when allylamine and ferrocene carboxaldehyde react in bulk solution and the product is monitored at a clean platinum electrode. The current observed for the reacted electrode decreased in amplitude slightly when the differential pulse scan was repeated; but, after the first few repetitions, stable and reproducible currents were obtained.

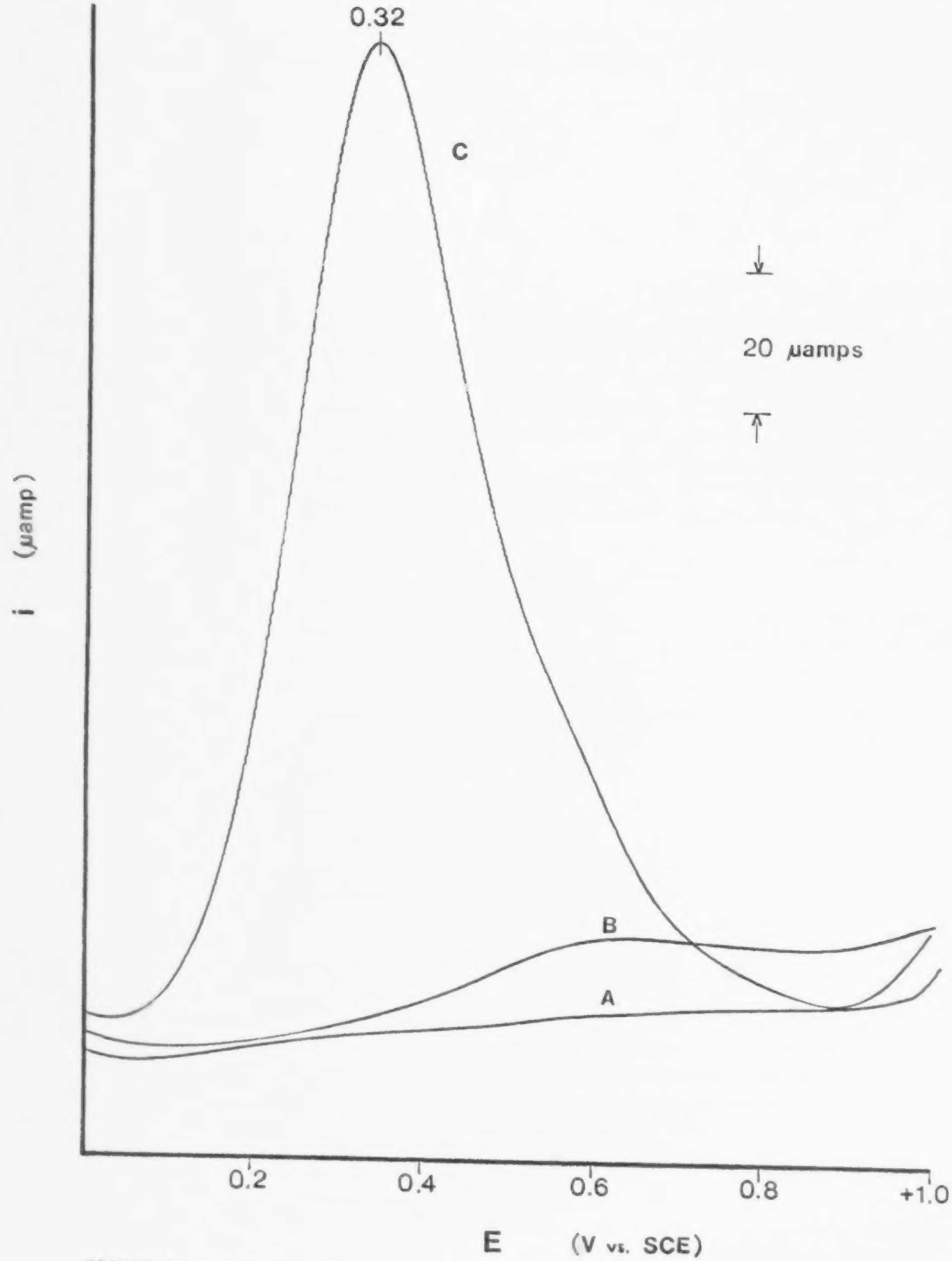


FIGURE 1 Differential pulse voltammograms of a clean platinum electrode (A), an electrode coated with allylamine (B), and an electrode coated with allylamine and reacted with 4.5×10^{-3}M ferrocene carboxaldehyde (C). Added uncompensated resistance: 1100 ohms.

RESULTS

By analogy to the conventional ASV technique, it is expected that the amount of ferrocene carboxaldehyde-allylamine condensation product formed at the CME surface as described above should be dependent on two experimental parameters: the concentration of ferrocene carboxaldehyde in the reacting solution and the reaction time allowed. Each of these effects was evaluated separately.

When identical allylamine-coated platinum electrodes were immersed for five minutes in reaction solutions containing a range of ferrocene carboxaldehyde concentrations, the series of differential pulse voltammograms shown in Figure 2 were obtained. For ferrocene carboxaldehyde concentrations of 10^{-2}M and above, the voltammograms were virtually identical, indicating similar and probably saturated surface coverage for each case. At concentrations less than 10^{-3}M, the peak current decreased gradually with concentration - with a detectable signal still observed at 10^{-7}M.

The level of response obtained for low analyte concentrations may be compared with data obtained using conventional electroanalytical techniques. Since ordinary differential pulse voltammetry is generally recognized as one of the most sensitive polarographic methods for bulk solution analysis, this technique was selected for the comparison. Accordingly, data compiled using the conventional differential pulse method (with the pulse height and scan rate chosen to match those used in the CME pre-concentration and stripping approach) are compared in Table 1 with data obtained using CME's. At high ferrocene carboxaldehyde concentrations, the conventional differential pulse signal is substantially larger than the CME response whose amplitude is limited at all concentrations by the finite quantity of analyte which it is possible to attach to the electrode surface. The differential pulse signal, however, depends on the diffusion of the electroactive species to the electrode and thus decreases rapidly with concentration. Hence, at low concentrations, the pre-concentration and stripping method can be expected to provide competitive performance. Its observed detection limit of 10^{-7}M compares very favorably with that seen for the conventional technique.

The voltammograms in Figure 2 represent behavior observed when allylamine-coated electrodes were allowed to react for a fixed time period in solutions of various ferrocene carboxaldehyde concentration. When the coated electrodes were immersed in identical 5×10^{-4}M analyte solutions and allowed to react for increasing time intervals, subsequent differential pulse analysis indicated that the +0.32 volt peak was formed very rapidly upon immersion. However, prolonged reaction times beyond about five minutes did not lead to increased ferrocene carboxaldehyde concentration at the electrode surface or to the increased peak currents that were expected. Rather, the peak height decreased gradually with time; and the peaks became distinctly broader in shape. Whether this observation is due to the further reaction of

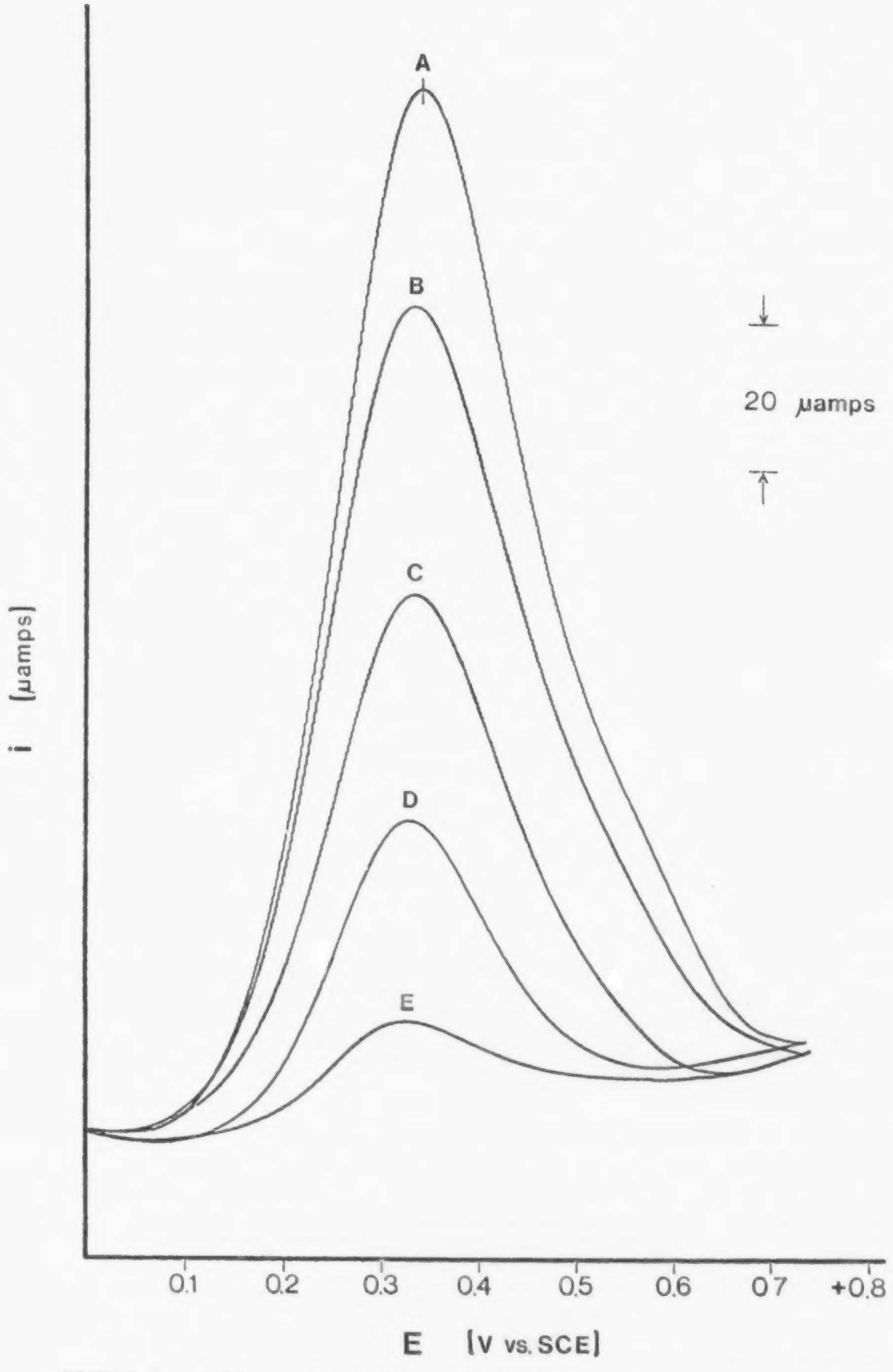


FIGURE 2 Differential pulse voltammograms for CME reacted with the following ferrocene carboxaldehyde concentrations: (A) 4.5 x 10^{-3}M; (B) 9.0 x 10^{-4}M; (C) 4.5 x 10^{-4}M; (D) 9.0 x 10^{-5}M; and (E) 1.1 x 10^{-5}M. Added uncompensated resistance: 1100 ohms

TABLE 1

Comparison of CME Response with Conventional Differential Pulse Voltammetry

[Ferrocene Carboxaldehyde], M	ip, CME, μamps[a]	ip, Bulk, μamps[b]
10^{-2}	120	-
10^{-3}	110	510
10^{-4}	41	60.5
10^{-5}	10	6.5
10^{-6}	6	0.73
10^{-7}	2	0

a. Reaction time - 5 min.

Reaction temperature - 75°C

E_p = +0.32 volts vs. SCE

Added uncompensated resistance: 1100 ohms

b. E_p = +0.61 volts vs. SCE

the ferrocene or imine moieties or to the gradual alteration of the CME surface under the reaction conditions employed has not yet been determined.

Finally, the selectivity of the concentration procedure was examined by allowing the imine formation to take place in the presence of both equimolar and hundredfold excess amounts of ferrocene itself. The voltammograms obtained under these circumstances were similar to those seen for ferrocene carboxaldehyde when no ferrocene was present, and the results are summarized in Table 2 . It is interesting to note that no drastic effect due to ferrocene was observed except for a slight suppression of the imine peak current at very high interferent concentrations. Apparently, the concentration reaction is selective for the carbonyl functionality, permitting the selective reaction and quantitation of samples containing this group in the presence of highly concentrated and otherwise identical species.

CONCLUSIONS

What we have described and tentatively evaluated here is a completely new approach for the trace level analysis of selected families of organic compounds. Its principal advantages appear to be three in number:

1. Sensitivity. The technique described is analogous in many respects to that of anodic stripping voltammetry, one of the most sensitive analytical methods. ASV is able to achieve detection limits in some cases below the ppb level primarily because of a pre-concentration step similar to that which we propose to employ. For the model analyte considered here, a detectable signal was observed at the 10^{-7}M or 25ppb level.

2. Selectivity. Conventional electrochemical methods possess sufficient selectivity of response to make them useful by themselves in some environmental applications. In addition to this selectivity based primarily on differences in analyte redox potential, our approach also offers a gross chemical selectivity for specific classes of compounds that derives from the nature of the pre-concentration reaction employed.

3. Convenience. Some existing analytical methods possess similar or greater selectivity and sensitivity than the proposed approach. But, of those which are useful for organic analysis, few offer these advantages at the same level of experimental convenience. Because of the selective pre-concentration step, the extraction or chromatographic step usually required prior to trace organic analyses might often be unnecessary. Additionally, the sensitivity obtained here utilized a pre-concentration time of only five minutes; and, as in most electrochemical methods, no sophisticated or expensive instrumentation was required.

TABLE 2

Effect of Ferrocene on CME Response to Ferrocene Carboxaldehyde

[Ferrocene Carboxaldehyde], M	[Ferrocene], M	ip, μamps
10^{-3}	0	104
10^{-3}	10^{-3}	96
10^{-3}	10^{-5}	102
10^{-5}	0	11
10^{-5}	10^{-3}	8
10^{-5}	10^{-5}	9
0	10^{-3}	0

Reaction time - 5 minutes

Reaction temperature - 75°C

Ep = +0.32 volts

Added uncompensated resistance: 1100 ohms

ACKNOWLEDGEMENT

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A COMPUTER INTERFACED TOXICITY TESTING SYSTEM FOR SIMULATING VARIABLE EFFLUENT LOADING

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ABSTRACT

Water quality criteria and standards are based primarily on toxicity tests carried out with single chemicals whose concentration is as nearly constant as possible. In the "real world," however, organisms are exposed to mixtures of chemicals which usually have markedly fluctuating concentrations. The primary difficulties in simulating "real world" conditions in toxicity testing are: (a) a means of varying chemical concentration to fit a predetermined set of conditions, and (b) a system which is capable of tracking and recording the response of aquatic organisms to these variations and which is quantitative and suitable for cross correlations of dose and response. Mini- and microcomputer interfacing with a toxicity testing system provides a means of systematically varying the concentration of a test chemical or chemicals in a continuous flow system. The same computer can also be used for the data acquisition system to store the voluminous time-series biological response data necessary for cross correlations with variable chemical concentrations. A description of the apparatus, examples of its use, types of data generated, and data analysis are discussed.

INTRODUCTION

Nearly all toxicity tests upon which water quality criteria are based have been carried out in test containers in which the intent was to keep the concentration of the toxicant constant. The development of continuous flow apparatus was in large part a consequence of the failure of batch test containers to maintain constant concentrations of certain kinds of toxic materials. Despite all the difficulties involved in maintaining constant concentrations and the rarity of tests in which constancy has actually been accomplished, one should bear in mind that lack of variability was the desired goal. However, when one attempts to transfer laboratory data to the "real world," one finds that constancy is the exception, not the rule, and that fluctuations in waste quality and quantity may occur from day to day, hour to hour, or even minute to minute. In spite of this lack of correlation between laboratory toxicity data and "real world" situations, toxicity tests in which the toxicant levels fluctuate according to a predetermined scheme have received little attention. This is probably due to the problems involved with automation of the toxicant controlling machinery. Consequently, applying results from relatively constant tests carried out in the laboratory to fluctuating "real world" situations presents various difficulties. Organisms are often apt to respond to thresholds rather than averages. On the other hand, duration of exposure is an important factor in determining a response. Some thresholds, therefore, may not elicit a response if the duration of exposure is too short. Water criteria and standards (for example, National Academy of Sciences (12)) have been developed for years without substantial attention to the fluctuating exposure. Only circumstantial evidence of the effectiveness of such standards exists, and no substantial body of evidence on the relationship between fluctuating and constant concentration exposures exists for aquatic organisms. Standards developed without this information might accidentally be correct, but might just as easily be either over- or underprotective. If either of the latter situations exists, the environment may be damaged or excessive expenditures may be made for unnecessary waste treatment systems.

One basic question is: in a qualitatively fluctuating effluent discharge, when do the organisms respond to the peak concentrations as if they were constant at the peak level? Additional interesting questions are:

1. What amplitude and frequency of episodic exposures, individually and of short duration, appear to have no deleterious effects?

2. When there are sublethal responses to fluctuating concentrations, are the organisms capable of returning to normal and, if so, how much time does it take?

3. At what concentration is amplitude a more important consideration than frequency of fluctuation, and vice-versa?

4. If a large retention pond with thorough mixing and delivery of a constant concentration and quality of waste is possible and conditions permit variable releases, is a pulsed delivery of the same total amount on a daily basis more or less harmful to the exposed organisms than a constant delivery of waste?

5. Is there some way in which regulatory standards could be modified to permit occasional nontoxic pulses of short duration when large holding ponds are not available and batch processing makes pulses quite likely?

This short paper could not possibly address all these questions; however, the instrumentation making such tests feasible, one of the main obstacles to answering these questions and other related questions, can be discussed. Two major capabilities must be incorporated into the instrumentation used to solve problems: (a) a means of predetermining the amplitude and frequency of the changes in toxicant concentration, and (b) a means of recording on a regular or continuous basis the biological response to these changes so that correlations between dose and response may be achieved. A statistical analysis that will enable a quantitative evaluation of the data generated must also be developed.

The key to resolving the problem is the interfacing of minicomputers with biological systems. This would permit the type of control necessary to make specific alterations in toxicant concentration at prescribed times, a means of handling large volumes of biological data and storing it, and a capability for pre-programming the analysis of data so that this can be accomplished in a cost-effective efficient manner.

The apparatus described herein represents an initial attempt to meet some of these requirements. This apparatus represents one of a series of uses of minicomputers in the acquisition and analysis of biological data from this laboratory (1,2,4,10,14). A first attempt to resolve a problem is usually imperfect. Nevertheless, the apparatus and the accompanying results described in this paper provide compelling evidence that sufficient promise exists for resolving some or all of the problems discussed previously and for justifying continued research and development of the apparatus. It is relatively inexpensive, simple, requires primarily shelf items, and is within the reach of most industrial facilities and nearly all major industrial research laboratories. In addition, once the apparatus is in place and functioning, it should be possible for technicians to operate and maintain it.

THEORY

The electrical action potential of muscle movement in the branchial region of a fish during ventilatory or "breathing" activity produces an electronic signal. This signal can be detected using electrodes submerged at opposite ends of a fish holding compartment (Fig. 1). When this microvolt signal is amplified to the 4 to 10 volt range, it can be recorded physically on a strip chart recorder (Fig. 2) or electronically on magnetic tape with a minicomputer.

That the ventilatory behavior of a fish will change when various toxicants, even at sublethal concentrations, are introduced into the water has often been demonstrated (6,11,14). However, the use of this principle as a continuous water quality monitor has been handicapped by the huge potential volume of data. A single unstressed bluegill (Lepomis macrochirus) can produce as many as 100,000 ventilatory signals in a 24-hour period. Natural variability requires that many individual organisms be monitored simultaneously for any analysis to be statistically valid and to prevent false indications of environmental contamination. Most workers in the past have recorded ventilatory activity intermittently with strip chart recorders and then analyzed data by personal observation, a tedious procedure at best (3,13). A more efficient use of such data is the continuous accumulation of ventilatory data from a large number of individuals over a long period of time. The most versatile and efficient method of accomplishing this is interfacing the fish with a minicomputer.

APPARATUS

An earlier version of the system now being used to investigate the ventilatory responses of the bluegill to sublethal doses of heavy metals has been described by Thompson et al. (14). Cairns et al. (5) demonstrated that, with slight modifications, this system could be used with numerous species, both freshwater and marine.

Sensing Chambers

A fish holding chamber with a total of 16 individual 700 ml capacity monitoring compartments was constructed of plexiglas (Figs. 1 and 3). The bottom of this chamber was constructed of 6.35 mm transparent plexiglas. The ends and dividing baffles were of 3.18 mm transparent plexiglas, and the sides of each individual compartment were of 3.18 mm opaque white plexiglas. All joints were fused with methylene chloride.

The submerged electrodes were of type 304 stainless steel wire and were attached with silicone aquarium cement to the baffles at either end of the fish monitoring compartment. The electrodes were on the side of the dividing baffles opposite the fish to prevent contact between fish and electrode. Each electrode was formed in such a way that the wire never passed through the flow of water from any of the perforations in the dividing baffles (Fig. 1). The electrode wire was insulated from just below the water line, where the insulation was sealed to the wire with silicone cement to prevent water intrusion, to the barrier strip on the outside of the tank. The only exposed wire was below the water surface.

Since fish respond to visual stimuli and to vibrations in a manner similar to their response to chemical stimuli, the entire fish holding chamber was enclosed in a plywood isolation chamber. The supports of this enclosure were anchored in sand filled containers to protect against vibration (Fig. 3).

Water Delivery

Diluent was dechlorinated tap water maintained at a constant temperature of 22°C. This was delivered by gravity flow to three plexiglas mixing chambers where the diluent could be mixed with various toxicant solutions if desired (Fig. 3). The water was then passed into delivery chambers, also of plexiglas, equipped with 1 mm diameter standpipes. A constant head pressure was maintained over these standpipes by delivery in excess and the excess was drained through an adjustable standpipe. This allowed delivery of water or water and toxicant at a constant rate and concentration to each testing compartment (Fig. 3).

Data Accumulation

Each monitoring compartment was connected to an amplifier specially designed for fish ventilatory investigations (8). These amplifiers were interfaced to a PDP-8E minicomputer through a 16-channel multiplexed analog to digital converter. Data were accumulated for 15-minute periods, printed on a Decwriter II computer terminal, and stored on magnetic tape cassettes by a Decassette TU60 tape drive. These data subsequently could be transcribed by the minicomputer over a telephone line to the Virginia Polytechnic Institute and State University's main computer (an IBM/370-1580) for analyses.

Computer Program

Although it is not apparent in Figure 2, the ventilatory signal is often bimodal (7,14). This bimodality is an artifact of the amplifier design and is more pronounced in more slowly ventilating individuals (14). The signal has been visually correlated with the ventilatory activity of *L. macrochirus*, and each waveform, bimodal or not, represents one complete ventilatory cycle (7). The computer program developed for this system is capable of rejecting this artifact when it is present and of accumulating the total counts as well as an approximation of the average amplitude of the signal. This program also uses a software controlled variable threshold to reject low amplitude noise. Rejection of real signals is less than 2 percent, while extraneous counts are less than 1 percent.

Toxicant Delivery

An Integral Masterflex, variable speed, peristaltic pump drive with remote control capability was used to introduce the toxicant into the mixing chambers (Fig. 3). As many as nine pump heads could be used simultaneously for delivery of different toxicant solutions to each mixing chamber or for producing various combinations in the mixing chambers. The toxicant solutions were pumped through 1.66 mm inside diameter silicone tubing. One of the three mixing chambers was used for control and, thus, never received toxicant solution.

The minicomputer used a digital to analog converter to deliver a preselected voltage to the pump drive. A computer-pump interface was used to convert the output voltage from the computer to the optimum range for use by the pump (Fig. 4). The computer output voltage could be changed by the computer at preselected one-hour intervals to simulate fluctuating "spills" or releases. The concentration of toxicant in the individual sensing compartments reached maximum concentration in 15-20 minutes, depending on the flow rate through each compartment.

TESTING PROCEDURE

Three tests were run to test the effect of Cu^{++} on the ventilatory activity rates of the bluegill. During each test, 11 fish received applications of $CuCl_2$ solutions, and 5 were left untreated as controls. The fish were maintained under conditions of continuous light to reduce diurnal variation. The fish were not fed while in the chambers or for 24 hours before being placed in the chambers.

The fish were allowed 24 hours in the testing compartments to acclimate before ventilatory signals were recorded. Ventilatory rates were recorded then for a total of 216 hours (9 days). No toxicant was applied during the first 96 or the last 24 hours. Toxicant solutions were applied in various patterns during the intervening 96 hours. During the first test, the toxicant was delivered at a constant rate for 96 hours at a concentration of 0.5 mg Cu^{++} per liter (Fig. 5). During the second and third tests, approximately equivalent amounts were delivered in intermittent patterns. The toxicant in test two was delivered during two variable 20-hour applications separated by a 20-hour recovery period (Fig. 6). The resulting concentrations of Cu^{++} were 0.5, 1.0, and 1.5 mg/l in 4-hour increments. Toxicant during run three was delivered in eight 5-hour applications with 1 hour increments (Fig. 7). The average concentrations during the 1-hour increments were 0.5, 1.1, and 1.3 mg/l. The variation in concentration is due to slight fluctuations in the voltage delivered to the pump by the computer.

DATA ANALYSIS

The total counts from four consecutive, 15-minute reporting periods were summed and divided by 60 to produce the average rate per minute for each 1-hour period for each individual fish being monitored. These average rates were graphed for the entire 216 hours of the test. Examples of these graphs are in Figures 5, 6, and 7.

While it is true that consecutive hourly averages for a single individual are not independent observations and that there is considerable natural variation that makes comparison between two individuals difficult, the application of a conventional one-way analysis of variance to data with large sample sizes is valid. This method was used to examine the differences between the mean rates of the combined controls and the three treatment patterns. For the purpose of the analyses the various patterns are indicated as follows. Pattern 0 = controls, all three tests combined; pattern 1 = one toxicant application; pattern 2 = two applications; and pattern 3 = eight applications. Separate analyses were performed on the pretreatment, treatment, and post-treatment data. Subsequently, a post-mortem Duncan's multiple range test was also carried out to locate possible sources of major differences.

The average rate for an entire period was calculated for each fish, and the analyses were run with these averaged data. The period for pretreatment was always 96 hours. The time for treatment was from the start of the first toxicant application until the end of the last application and varied from run to run. The time for the post-treatment period was all the remaining time after toxicant delivery ceased and was also variable.

RESULTS

Five of the 48 fish are not included in the final analysis due to various physical and electronic failures. Among the remaining 30 individuals that received toxicant in 1 of the 3 patterns, 27 showed an average increase of 16.4 counts per minute during the treatment period. The 3 that decreased did so by an average of 1.4 counts per minute. Among the 13 control fish, 8 increased by an average of 3.9 counts per minute, and 5 decreased by an average of 3.5 counts per minute.

After the toxicant flow ceased, most of the treated individuals responded with reduced ventilatory rates, and 29 of the treated fish survived the treatment period. Four of these had increased counts averaging 3.4 per minute, and 25 decreased by an average of 17.0 per minute. Among the

13 untreated controls, 3 increased by an average of 2.2 counts per minute, and 10 decreased by a 5.7 count average.

The analyses of variance indicated that there was no statistical difference between the controls and the treated individuals during either the pretreatment or the post-treatment periods; the corresponding probabilities for no difference were 30 percent and 9 percent (Table 1). Thus, the null hypothesis was acceptable at the 0.05 alpha level (Table 1). During the treatment period, the probability for no difference was 4 percent, indicating rejection of the null at the 0.05 level.

Duncan's multiple range test indicated no difference between controls and any other fish during the pretreatment period. During the treatment period, there was no detectable difference between any of the three treatment patterns. The controls, however, could not be differentiated from pattern 3, but they could be separated from patterns 1 and 2. However, it should be noted that these analyses are based on the average rate for the entire period from the start of the first toxicant application to the end of the last application, including the intermittent periods of no toxicant application. It is quite apparent from Figure 7 that there is definitely a noticable reaction in some of the individuals. That this does depend to some extent on the pattern of application can be noted from Figures 5, 6, and 7. The results of Duncan's test for the post-treatment period gave mixed results also. The treated fish from run one (pattern 1) could not be distinguished from the controls, neither could the treated fish from run two (pattern 2) or three (pattern 3) be distinguished from the controls. However, these could be differentiated from those treated with pattern 1 (Table 1).

DISCUSSION

Some tentative conclusions can be drawn from this work even though any final conclusions must await a larger data base.

1. There is a trend toward a reduction in the average rate of ventilatory activity over time. This is probably due to the fact that the fish are not fed while in the sensing chambers.

2. The presence of cupric chloride in solution at sublethal levels causes a general increase in the ventilatory activity of most bluegills which can be statistically significant if the sample size is large enough.

3. With the analyses used, differences between treated and untreated fish can be detected, but differences between application patterns cannot. However, the response to the different patterns can be distinctive as can be determined from visual inspection of the graphed data.

4. There was apparent recovery with respect to ventilatory rate in many individuals within 24 hours after

toxicant delivery ceased. However, long-term chronic effects cannot be implied from these data at present.

5. The apparatus provided the type of information it was designed to produce and merits additional attention.

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TABLE 1

Results of Analysis of Variance (ANOVA)

	Pretreatment period			Treatment period			Post-treatment period		
	F	P	df	F	P	df	F	P	df
ANOVA Results	1.26	0.30*	3,39	3.16	0.04	3,39	2.29	0.09*	3,38
Duncan's** Test Results	0 = 1 = 2 = 3			1 = 2 = 3, 0 = 3			1 = 0, 0 = 3 = 2		

* Null hypothesis may be accepted at the 0.05 α level.

** 0 = controls, 1 = continuous application, 2 = 2 applications, 3 = 8 applications.

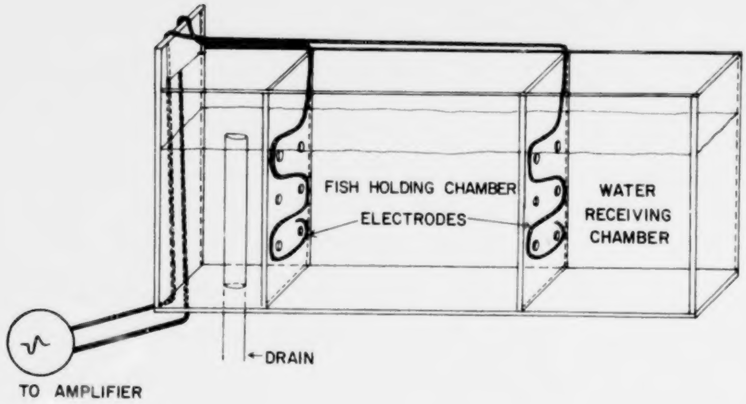
FISH HOLDING CHAMBER
ELECTRODES
WATER RECEIVING CHAMBER
DRAIN
TO AMPLIFIER

FIGURE 1 Individual monitoring compartment.

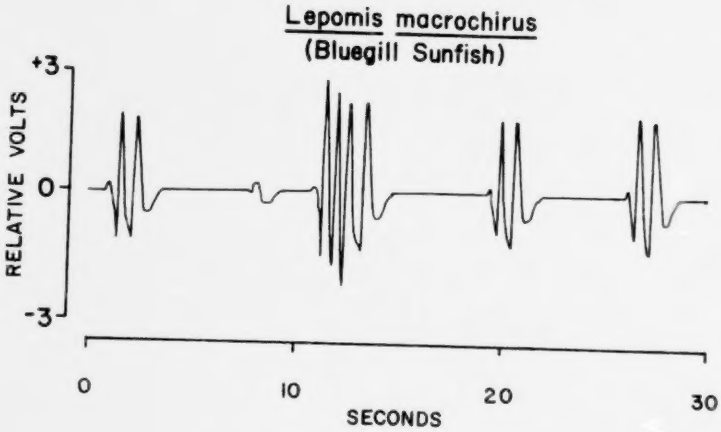

FIGURE 2 An example of the ventilatory signal as recorded on a strip chart recorder.

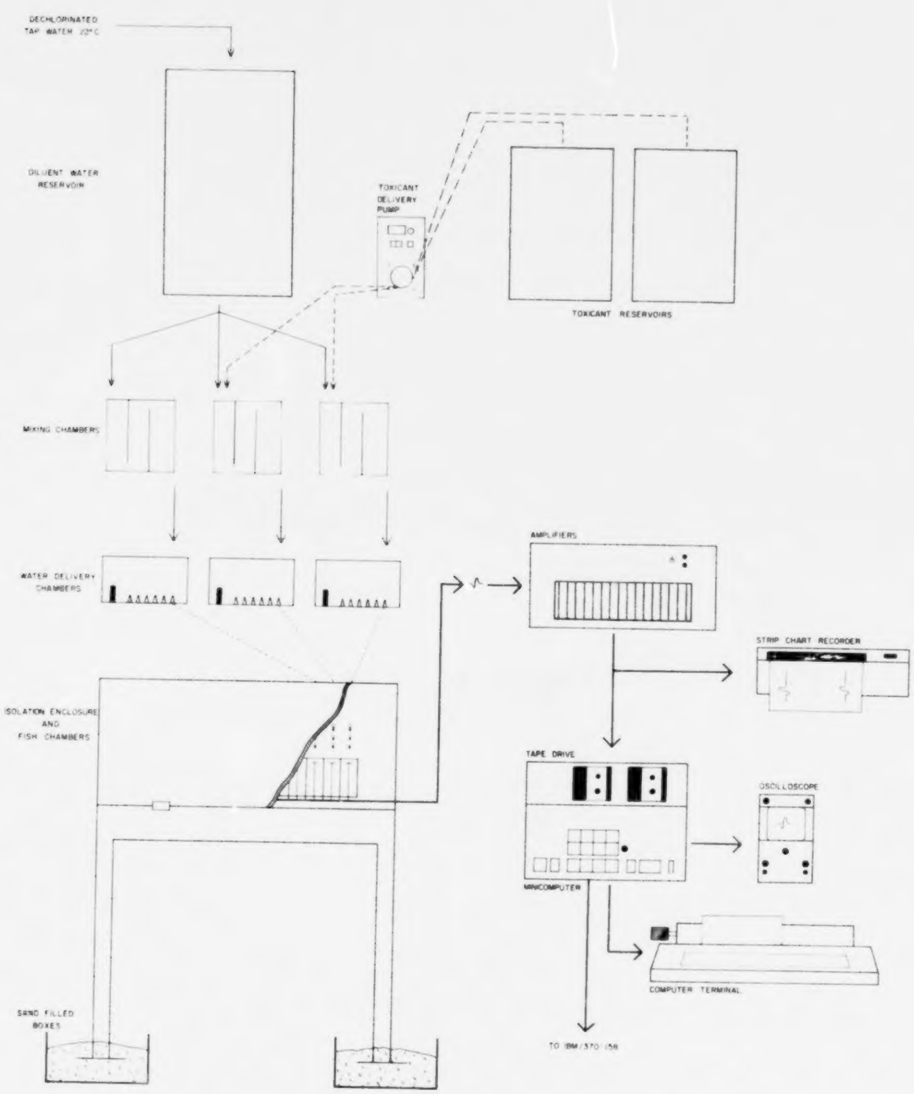
DECHLORINATED TAP WATER 22°C
DILUENT WATER RESERVOIR
TOXICANT DELIVERY PUMP
TOXICANT RESERVOIRS
MIXING CHAMBERS
WATER DELIVERY CHAMBERS
AMPLIFIERS
STRIP CHART RECORDER
ISOLATION ENCLOSURE AND FISH CHAMBERS
TAPE DRIVE
OSCILLOSCOPE
MINICOMPUTER
COMPUTER TERMINAL
SAND FILLED BOXES
TO IBM/370 158

FIGURE 3 The water delivery and data accumulation system.

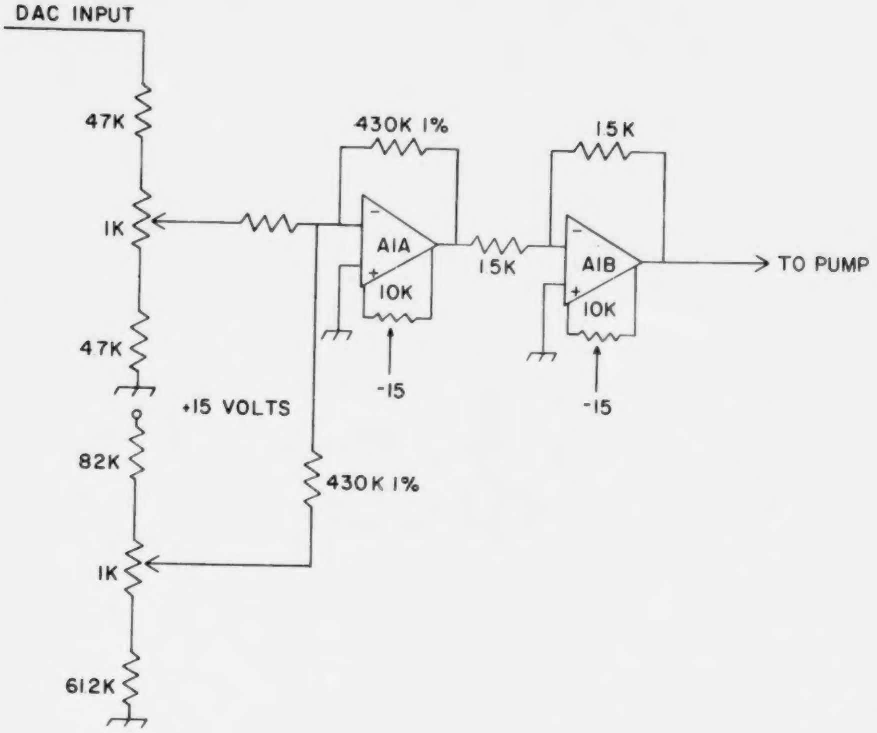
DAC INPUT
47K
1K
47K
+15 VOLTS
82K
1K
612K
430K 1%
430K 1%
A1A
10K
-15
15K
15K
A1B
10K
-15
TO PUMP

FIGURE 4 Schematic for the computer/pump interface (redrawn from Landers (9)).

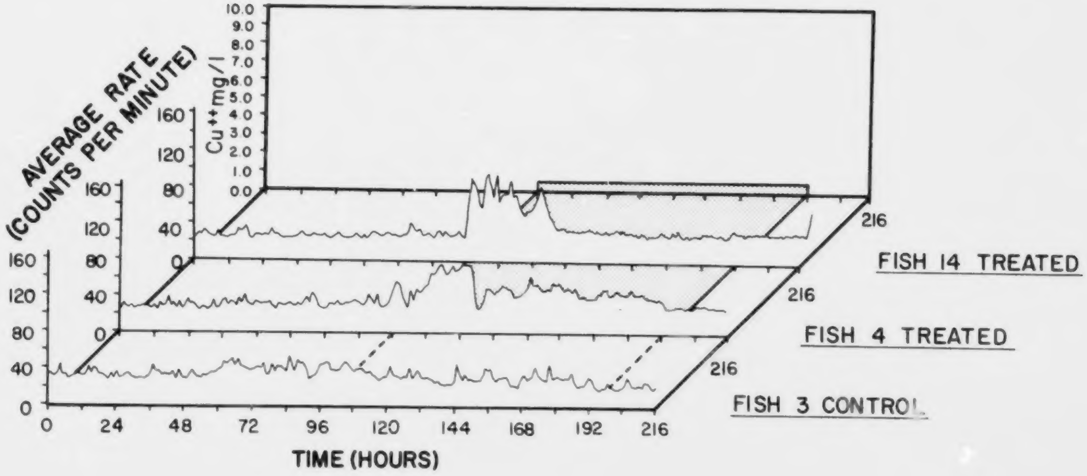

FIGURE 5 Examples of the average ventilatory rates from the first test. The individual on the first plane received no toxicant application while those on the second and third planes received 0.5 mg/l of Cu^{++} during the treatment period (shaded area). The sudden increase in ventilatory rate at the start of the treatment with a gradual reduction by the end of the treatment period was typical of most of the individuals receiving the toxicant in this pattern.

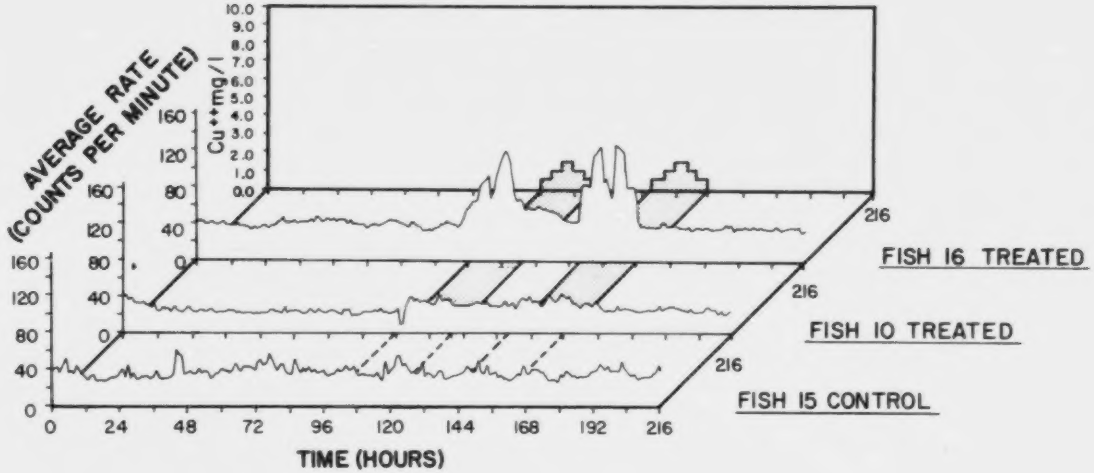

FIGURE 6 Examples of the average ventilatory rates from the second test. The treated fish received toxicant in two doses at levels of 0.5, 1.0, and 1.5 mg/l Cu^{++}. Note the variation of response among the treated fish. The two treated fish in this figure represent the extremes found among the treated individuals. The apparent reduction in rate at the peak of the second toxicant application is due to another reaction to the toxicant, a reduction in the amplitude of the ventilatory signal. While the rate probably remained in the range of 120 counts per minute, the amplitude dropped below the limit of detection. This reaction is currently being studied and will be reported in more detail at a later date.

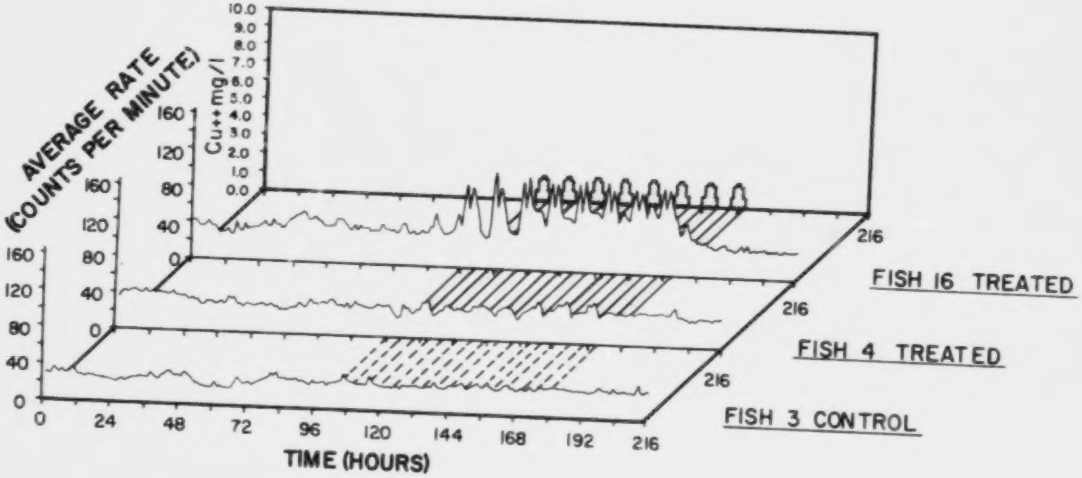

FIGURE 7 Examples of the average ventilatory rates from the third test in which the toxicant was applied in eight short bursts. The concentrations achieved were 0.5, 1.1, and 1.3 mg/l Cu^{++}. Note the variation of response among the treated fish. The two treated fish in this figure represent the extremes found among the treated individuals. The apparent reduction in rate at the peak of the second toxicant application is due to another reaction to the toxicant, a reduction in the amplitude of the ventilatory signal. While the rate probably remained in the range of 120 counts per minute, the amplitude dropped below the limit of detection. This reaction is currently being studied and will be reported in more detail at a later date.

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ON-LINE MONITORING OF TOXIC MATERIALS IN SEWAGE AT THE LAWRENCE LIVERMORE LABORATORY

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ABSTRACT

It is becoming increasingly important for industry to prevent releases of potentially toxic material to the environment. The Lawrence Livermore Laboratory, a U.S. Department of Energy research facility, has developed a system to monitor its sewage effluent on a continuous basis.

A representative fraction of the total waste stream leaving the Plant is passed through a detection assembly consisting of an x-ray fluorescence unit which detects high levels of metals, sodium iodide crystal detectors that scan the sewage for the presence of elevated levels of radiation, and an industrial probe for pH monitoring. With the aid of a microprocessor, the data collected is reduced and analyzed to determine whether levels are approaching established environmental limits. Currently, if preset pH or radiation levels are exceeded, a sample of the suspect sewage is automatically collected for further analysis, and an alarm is sent to a station where personnel can be alerted to respond on a 24-hour basis. In the same manner, spectral data from the x-ray fluorescence unit will be routed through the 24-hour alarm system as soon as evaluation of the unit is complete.

The monitoring system has played an important role in averting treatment plant problems when a spill has occurred. The design of the system and operational experience will be discussed.

INTRODUCTION

Since its beginning in 1952, the Lawrence Livermore Laboratory (LLL), Livermore, California has supported an environmental surveillance program to determine its impact, if any, on the local environment. The Laboratory, located in a suburban valley 65 km east of San Francisco, is involved in widely varied research and development programs for the U.S. Department of Energy. The main efforts are in nuclear and nonnuclear weapons, magnetic and laser fusion energy, biomedical research, and nonnuclear energy technologies, such as geothermal power and fossil fuel utilization. These programs and the support efforts involved in carrying them out generate waste products that could present a negative impact on the environment if not properly managed. Although the Laboratory has an extensive program to control and dispose of all potentially toxic materials at the source, the environmental monitoring program serves as a check on control procedures. An important part of this monitoring program is the on-line wastewater monitoring system, which detects elevated radiation, metal concentration, and pH levels.

BACKGROUND

The Laboratory's sanitary sewer system is a possible route for the escape of toxic materials. Its effluent, which is approximately 350,000 gallons per day, is processed by the municipal Livermore Water Reclamation Plant. The plant is a secondary treatment operation, whose output is discharged to several different sources. Until very recently, most of this effluent was returned to a creek which eventually recharged a groundwater basin. A portion of the effluent is used for irrigating vegetation along roadways and a few local crops. Earlier this month, however, a transport pipeline to the San Francisco Bay was completed, and most of the treated effluent now flows to the Bay. The sewer monitoring system was designed and constructed to detect toxic material releases and to facilitate immediate response action when they occur. This is necessary in preventing damage to the city treatment plant and regions receiving discharged effluent. If toxic materials are detected at levels that exceed predetermined LLL alarm limits, signals are sent to a central alarm station that is manned 24-hours a day and a sample of the suspect toxic effluent is automatically collected. Since two hours pass before LLL effluent reaches the treatment plant, sufficient time is available to alert emergency personnel, evaluate the situation and, if necessary, arrange for diversion of the material to emergency holding basins at the treatment plant. The toxic waste can be treated in the basins or removed without destroying or cutting down on the efficiency of the treatment plant.

MONITORING SYSTEM COMPONENTS

The on-line monitoring system consists of several components. The flow route to these components starts at a point in a manhole where all Laboratory sewage discharge lines converge. As the sewage flows through a Parshall flow-measuring flume, approximately 40 L/min of it is pumped to an above ground building where the detection instrumentation is located. Inside this building, the sample enters a tank housing the high- and low-energy radiation detectors, pH probe, and a sampling line leading to the metal analyzer unit. After it is scanned, the sample is returned to the sewer via an outlet pipe.

Radiation detectors

Radioisotopes being used at LLL that could be released in quantities that would exceed continuous exposure maximum permissible concentration (MPC) levels are ^{90}Sr, ^{235}U, ^{238}U, ^{237}Np, ^{238}Pu, ^{239}Pu, ^{241}Am and ^{244}Cm. All of these, with the exception of ^{90}Sr, emit heavy element x-rays and low energy gamma rays during decay. For ^{90}Sr, low energy bremsstrahlung photons give an indication of specific activity. With this in mind, the radiation detection system was designed (1). The primary detector, a 3 x 127 mm sodium iodide crystal separated from the sample by a thin polycarbonate plastic sheet, allows recognition of the low energy x-rays, gamma rays, and bremsstrahlung photons in the energy range of 10 to 100 keV. In addition, a 50 x 50 mm sodium iodide crystal detector is mounted immediately adjacent to the sample tank to provide detection of higher energy (100 - 1000 keV) events.

The electronics associated with the detectors includes single channel analyzers and an Intel 8008 microprocessor which analyzes the data and decides whether preset levels have been exceeded. The alarm system for radiation detection has been set at a level that corresponds to less than 2% of the allowable monthly discharge limit for a continuous release of ^{90}Sr or less than 0.5% of the monthly allowable discharge limit for a continuous release of ^{239}Pu (2).

pH monitor

A commercial industrial pH probe is housed in the tank holding the radiation detectors. Output from the pH sensor is sent to the Intel 8008 microprocessor where the data is evaluated. If the pH drops below 3 or exceeds 11 for five minutes within a "floating" ten minute block of time, the alarm system is activated.

Metal detection

The deleterious effect of excess concentrations of heavy metals on sewage treatment plant operations has been demonstrated in a number of studies. The Laboratory has had two incidents in the past when inadvertent releases of toxic metals have created operating problems at the sewage treatment plant. In one incident, copper cyanide solution destroyed 50% of the bacteria in the trickling filter unit, and in the other, excess chrome significantly reduced the efficiency of one of the two digestor units. These events prompted a search for a continuous metal detection system that would prevent the recurrence of plant down-time caused by a metal release to the sewer. When the search was started about two years ago, commercial units available appeared to be unsuitable for sewage analysis. The units required pre-analysis filtering, which would affect the representability of the sample and which would require a considerable amount of maintenance. A monitoring unit capable of detecting hazardous concentrations of ions found to be most harmful to the bacteria in the treatment plant process, specifically copper, nickel, chrome and zinc was then designed. The system meets the requirements placed on a continuous system: it is fairly reasonable in cost, especially when contrasted to the cost of reseeding a treatment plant; works on-line and in real time; does not require extensive pretreatment of the sample, thereby preserving representability; and requires minimal maintenance.

The unit providing metals detection is an x-ray fluorescence analyzer (XRFA). Its design is based on the principle that elements emit characteristic x-ray lines when excited by a radiation source. These x-ray lines can be measured by energy dispersion techniques to determine their energy, which permits species indentification, while the intensity of the lines is proportional to concentration.

A portion of the sample stream that flows through the tank described previously is routed through a macerator to reduce any solids to particles 100 microns or less in diameter. The flow is then introduced through a nozzle into a flume inclined at 45°. The flow spreads to a sheet 1 mm in depth before it reaches the source/detector region of the unit. A thin plastic window backed by air is positioned under the stream in this region to prevent detection of the chrome and nickel in the stainless steel flume. A ^{109}Cd source is used to excite the elements of interest if they are present in the stream, and a commercial xenon-CO_2 mixture x-ray proportional counter is the detector used. The output from the detector goes to amplifiers, then through an analog-to-digital converter interfaced to a Digital Equipment Company LSI-11 microprocessor.

A dual register system for the data output allows both a rapid response to high metal concentrations (500 sec count) and a more sensitive response to low concentrations released over a longer time frame (5000 sec accumulated count). The data counting system also provides for advisories at concentrations that are elevated, but not at alarm levels. Minimum advisory and alarm levels are shown in Table 1.

Maximum permissible discharge concentrations for copper, chrome, nickel, and zinc have been established at LLL based on studies of the effects of toxic metals on sewage treatment and the dilution factors resulting from the intermixing of LLL's effluent with the domestic sewage from the Livermore city population. These concentrations, as well as the 500- and 5000-sec alarm limits for the elements of concern, are shown in Table 2. Although only zinc will cause an alarm in 500 sec if its LLL limit is exceeded, copper, chrome and nickel will cause alarms in 5000 sec or less if their limits are exceeded. This will provide adequate response time.

System monitor

In addition to detecting high- and low-energy radiation, pH excursions, and heavy metal releases, the monitoring system trips an alarm when an equipment malfunction occurs. A signal is sent to the central alarm station if problems such as a count rate lower than background, power failure, or flow malfunction occurs.

Grab sampler

An important part of the monitoring system is a mechanism that extracts a sample from the waste stream as soon as an alarm is tripped. This sample is often valuable in identification of the toxic material involved and in the evaluation of the situation.

TABLE 1

Minimum Advisory and Alarm Levels for the XRFA Monitor

Element	500 sec		5000 sec	
	Advisory, ppm	Alarm, ppm	Advisory, ppm	Alarm, ppm
Cr	36	119	11	37
Mn	23	78	7	25
Fe	15	50	5	16
Co	10	33	3	10
Ni	6	21	2	7
Cu	5	16	2	5
Zn	4	14	1	4
As	2	7	1	2
Se	2	7	1	2
Hg	5	17	2	5
Pb	4	12	1	4

TABLE 2

Comparison of LLL Maximum Permissible Metal Discharge Limits and XRFA Alarm Limits

Single metal	LLL limit, ppm	XRFA alarm limit 500 sec, ppm	 5000 sec, ppm
Cr	100	119	37
Cu	10	16	5
Zn	50	14	4
Ni	10	21	7

Combinations: total concentration to be less than 100 ppm

Teletype printouts

Both the LSI-11 and Intel 8008 are interfaced to teleptypes. The printouts provide responding personnel with current as well as historical data on effluent releases.

OPERATING EXPERIENCE

For the most part, the existing monitoring system has proved to be very effective. Since it has been in operation, there have been several occasions when diversion of the Laboratory's flow at the Livermore treatment plant was deemed necessary. In all instances, alarms were set off because of inadvertent acid releases. These incidents alone have proved the worth of the system since restoring the treatment plant to normal operation would have been more costly than the investment made in designing, constructing, operating and maintaining the monitoring system.

In addition to the pH excursions, there have been a number of radiation alarms. In all instances, grab samples analyzed have shown that the material triggering the alarm was a radiopharmaceutical, generally ^{99}Tc from brain scans or ^{131}I from thyroid treatments. These alarms are caused by the radiopharmaceutical being excreted by outpatients who return to work following medical treatment.

Although the alarm system has not yet been hooked up for the x-ray fluorescence analyzer, an inadvertent release of about 40 ppm chrome from a plating operation was noted on the teletype printout.

SUMMARY AND CONCLUSIONS

The on-line sewer monitoring system cannot in itself prevent the accidental discharge of toxic materials into sewers. However, by rapidly detecting releases and sounding alarms to alert emergency personnel to respond, actions can be taken to prevent damage to the environment. Because it is a real-time system, Laboratory personnel can more easily locate the source of a toxic discharge and take corrective action to prevent its recurrence.

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COMPARISON OF FOUR LEACHATE GENERATION PROCEDURES

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ABSTRACT

Four leachate procedures, ASTM Method A, ASTM Method B, EPA-OSW Extraction (EP), and Carbonic Acid Extraction (CAE), have been evaluated for their general applicability, reproducibility, Environmental Assessment methods compatibility, and leaching power. The leachates generated by these methods were analyzed for nine metals by atomic absorption methods and for F^-, $C\ell^-$, and SO_4^{2-} by ion chromatography. Seven energy process wastes including oil shale, FBC waste, fly ash, boiler slag, scrubber sludge, and hopper ash were extracted to evaluate the general applicability of the leachate tests. The ASTM methods had the best reproducibility, while the EP had the poorest precision. The EP and CAE procedures leached the largest quantities of trace metals from the wastes. However, based on the total metal concentration in the sample, the leachate methods generally extracted < 1%. The EP and ASTM-B methods caused some difficulty with flameless AA analyses. Based on the RCRA criteria, five of the energy wastes would be classified as hazardous by at least one of the leachate procedures. Selenium usually exceeded the threshold value for the leachate.

INTRODUCTION

In order to fulfill the solid waste characterization needs of Environmental Assessment (EA) programs, the Process Measurements Branch of EPA/IERL-RTP has directed research to identify a suitable leachate generation procedure. In conjunction with this effort, the GCA/Technology Division has evaluated four leachate generation methods. The evaluations include the following methods as described:

- EPA-OSW Extraction Procedure (EP) - an open system, acetic acid extraction;
- ASTM Method A (ASTM-A) - a closed system, water extraction;
- ASTM Method B (ASTM-B) - a closed system, acetic acid-acetate buffer extraction;
- Carbonic Acid Extraction (CAE) - a closed system, CO_2-saturated water extraction.

These procedures are discussed briefly below. The experimental parameters for these methods are compared in Table 1.

The Extraction Procedure (EP)

The EP method has been proposed by the EPA-OSW to meet the RCRA guidelines in evaluating the hazards of solid waste disposal. With the addition of acetic acid to the aqueous solution, the procedure presumably intends to simulate the first stage of anaerobic degradation, involving the formation of volatile, organic acids at a disposal site. The acidic medium also provides a more aggressive leaching test than the purely aqueous leachate.

The experimental procedure suggests a minimum size of 100 grams for the extraction. The separation of any liquid fraction from the original sample is accomplished by filtration or centrifugation methods. After sample preparation, consisting of either grinding or subjecting the sample to the structural integrity test, the solid is placed in an extractor which must be capable of thoroughly mixing the solid and the leaching medium. A stirring device is suggested, but other agitation methods may also be used. An amount of deionized water equal to 16 times the weight of sample is added. The pH of the resulting leachate is monitored, maintained at 5.2 ± 0.2 and adjusted with 0.5 N acetic acid, if necessary. The extraction proceeds for 24 hours with a maximum addition of 4 mℓ of acid per gram of sample permitted to maintain the pH. After 24 hours, the mixture is filtered and deionized water is added to adjust the volume to 20 times the weight of the sample.

ASTM Methods

The American Society for Testing and Materials (ASTM) has proposed two procedures to determine the leachable components of a solid waste. Both methods are "intended as a rapid means of obtaining a solution for evaluation of the extractable materials in waste. They may be used to produce solutions for the estimation of the relative environment hazard inherent in the leachings from the waste". The wastes are to be used in the form which they are disposed. Where available, sampling is to proceed using ASTM sample methods developed for the specific industry.

TABLE 1

Comparison of Experimental Parameters for the ASTM-A, ASTM-B, EP, and CAE Leachate Methods

Parameter	ASTM-A	ASTM-B	EP	CAE
Leaching Medium	ASTM Type IV H_2O	Sodium Acetate - Acetic Acid Buffer	0.5N Acetic Acid	CO_2-saturated H_2O
Minimum Sample Size	350 g	350 g	100 g	100 g
Sample Preparation	None; Use in Disposed Form	None; use in Disposed Form	Grind or Subject to Structural Integrity Test	None; use in Disposed Form
Solid to Liquid Ratio	1:4	1:4	1:20	1:16
Agitation Method	Reciprocating Shaker Recommended	Reciprocating Shaker Recommended	Unspecified Extractor; Stirring Device Suggested	Reciprocating Shaker Recommended
Agitation Time	48 hrs.	48 hrs.	24 hrs.	48 hrs.
Initial pH of Leaching Solution	5.6 - 5.9	4.5	5.6 - 5.9	3.9 - 4.0
Number of Extractions	1	1	1	1
Temperature	Room	Room	Room	Room

The water extraction (ASTM Method A), uses Type IV water for the extraction, while the ASTM Method B employs an acetic acid-acetate buffer solution to leach the metals from the wastes. The experimental procedure, however, is identical for each method. A minimum sample size of 350 g is recommended for each method.

The quantity of sample chosen for leaching is placed in a round, wide-mouth bottle (constructed of material appropriate for the solid waste and subsequent analyses) and mixed with the H_2O or acetate buffer. The volume, in milliliters of leachate added, is equal to four times the weight in grams of the sample. Mixing of the phases is accomplished by any apparatus which is capable of producing the constant movement equivalent to a reciprocating shaker operated at 60 to 70 one-inch strokes per minute. Agitation is continued for 48 hours, followed by vacuum filtration of the liquid phase.

The Carbonic Acid Extraction (CAE)

The Carbonic Acid Extraction (CAE) was introduced as an alternative to the leaching media of the previous methods. The CO_2-saturated water was intended to simulate the aggressive leaching characteristics of the acetic acid and acetate buffer solutions without having the problems associated with the bioassay tests and analytical methods.

In order to maintain a CO_2-saturated leachate, the extraction must be performed in a closed system. Thus, the CAE followed the basic procedure and agitation method of the ASTM methods. A minimum sample size of 100 g is suggested for the extraction. Round, wide-mouth linear polyethylene bottles are used to contain the leachate. The liquid to solid ratio is 16:1 and was chosen to minimize common ion effects, which may affect the solubility of some species in the leachate. It was hoped that this liquid to solid ratio would be low enough to prevent the trace elements from being diluted below the AA detection limits.

The carbonic acid solution is prepared by bubbling CO_2 through the deionized water until the pH reaches a minimum (approximately 3.9 to 4.0). The mixture was agitated at the rate advocated by the ASTM methods (60 strokes/minute). After shaking for 48 hours, the leachates were vacuum filtered through a 0.45 μm filter.

Evaluations

The evaluations of the four leachate procedures were based upon the following criteria:

- General Applicability - Any procedure employed as part of EA methodology must be amenable to a wide range of waste materials;

- Reproducibility - In order to make valid judgements regarding the potential hazard of a waste, the reproducibility of the generation procedure must be well defined;
- EA Methods Compatibility - It is highly desirable that the leachate produced not necessitate modifications to the established analytical procedures;
- Leaching Power - To the extent practical in the laboratory, the leachate generation procedure utilized should simulate the anticipated fate of the waste.

RESULTS AND DISCUSSION

General Applicability of the Leachate Methods

To evaluate the general applicability of the leachate methods, the four procedures were applied to a variety of energy process wastes, including oil shale, fluidized bed combustion waste, bituminous coal fly ash, bituminous coal boiler slag, lignitic coal scrubber sludge, and hopper ash from a coal-fired power plant. Except for the hopper ash, the waste samples were supplied as part of an ASTM interlaboratory test program to assess three extraction procedures, the ASTM-A, ASTM-B, and EP methods. Most of these wastes were essentially dry, since no weight was lost when the percent moisture was determined for the ASTM methods. The only exception was the scrubber sludge, which had a weight loss of 28% upon drying. For the wastes tested, no procedural problems were encountered with any of the leaching methods. All of the samples were extracted in the form in which they were received. None of the wastes had to be ground or subjected to the structural integrity test, as prescribed in the EP method. Since the samples were basically dry solids, the preparation schemes of the leachate methods have not been tested thoroughly. For more complex industrial wastes, a protocol for liquid-solid separation may not be adequately addressed by some of the procedures.

Leaching Power

The final pH data for the leachates are summarized in Table 2, which also indicates the number of extractions completed for each sample and each leachate method. As indicated in Table 2, the reproducibility of the pH was quite good for replicate extractions by all four procedures.

TABLE 2

Summary of Final pH for Wastes Tested[*]

Waste Sample	ASTM-A	ASTM-B	EP	CAE
Oil Shale	9.88	5.09	8.70	6.64
	11.13[†]	5.32	8.50	6.58
	10.74[**]			
	10.74[**]			
FBC Waste	12.52	11.94	12.28	11.74
	12.54		12.32	
Bituminous Coal Fly Ash No. 1	10.4	4.5	5.0	
			5.0	
			5.0	
Bituminous Coal Fly Ash No. 2		3.28		3.11[‡]
		3.51[‡]		3.08[‡]
Bituminous Coal Boiler Slag	3.55	4.27		4.22[‡]
Lignitic Coal Scrubber Sludge	5.0	4.5	5.1	5.43
Hopper Ash	12.13	11.03	9.44	7.30
	12.16	11.04	10.37	7.25
	12.16	11.02	10.22	7.33

[*] Unless otherwise noted, the agitation rate for ASTM-A, ASTM-B, and CAE was 60 strokes/min.

[†] Sample leached with bottle lying horizontally on shaker.

[**] Sample leached with no agitation.

[‡] Agitation at 120 strokes/min.

The release of trace metals has been correlated with the pH of the aqueous extract. The desorption of trace metals from fly ash surfaces decreases with increasing pH. Additionally, fly ash has been shown to affect the pH of the aquatic environment.(9) The change in pH is a function of iron and/or calcium in the fly ash. The amorphous iron oxides produce an acidic solution while the lime ($Ca(OH)_2$) yields a basic extract in distilled water. For many of the wastes, the final pH of the H_2O extraction correlates with the predominance of Ca or Fe oxide. An exception to this is the bituminous coal fly ash No. 1 which gave a basic pH in distilled water although the major oxide was iron. For the FBC waste and hopper ash, the predominance of the CaO offset the acidic media of the ASTM-B, EP, and CAE leachates and produced a basic pH.

Some of the initial metals analyses were done by flame atomic absorption. This applies to the results for the bituminous coal fly ash No. 1 and the lignite coal scrubber sludge. Since many of the results for these leachates were below the flame AA detection limits, it was necessary to use the graphite furnace, with its greater sensitivity, for the AA analyses. Calcium was determined by flame AA while mercury was analyzed by the cold vapor technique. The anions, F^-, $C\ell^-$, and SO_4^{2-}, were determined by ion chromatography.

Typical leachate data for the FBC waste is shown in Table 3. To facilitate intermethod comparisons, the solution concentrations for the leachates were converted to micrograms of contaminant per gram of dry sample. The results indicate that the major components in the leachates are Ca^{2+} and SO_4^{2-}. The fluoride and chloride could not be determined in the ASTM-B and EP leachates because the acetate ion present in these leaching media interferes with the IC analyses. It appears that most of the trace metals solubilized during the extractions exist as sulfate compounds. These results are supported by the findings of a previous study.(7) The IC analyses of oil-fired and coal-fired fly ashes indicate that the predominant anion in solution was SO_4^{2-}. Fourier transform infrared analysis of the water-soluble fractions supported the assumption that the soluble metals like nickel, vanadium, and magnesium are sulfate forms.

As an aid to evaluating the leachate data, the number of times each leachate method gave the highest concentration or highest quantity (in mass/g of dry sample) of an inorganic contaminant is tabulated in Tables 4 and 5, respectively. The results were compared only for the four wastes extracted by all four leachate procedures. These four wastes were oil shale, FBC waste, lignite coal scrubber sludge, and hopper ash. When a waste was extracted more than once by a method, the results were averaged before comparing the data. In some cases, the results were below the detection limits of the analytical technique, and an intercomparison of the leachate tests was not made. This omission of some data sets is reflected in the number of comparisons made for each inorganic species (the maximum number of tests for each species would be 4). No comparisons were made for fluoride and chloride because these anions could not be analyzed in the leachates generated by the ASTM-B and EP methods.

TABLE 3

Comparison of Leachate Data (in µg/g)[*] for FBC Waste

Species	ASTM-A[‡]		ASTM-B	EP[‡]		CAE
Ca (mg/g)	3.8	4	10.8	56	56	4.3
Ag	<0.002	<0.002	0.002	<0.01	<0.01	0.010
As	0.15	0.14	0.25	0.17	0.13	0.40
Ba	<1.6	<1.6	0.22	7.2	7.7	1.1
Cd	0.002	0.002	0.068	0.004	0.018	<0.002
Cr	0.25	0.12	0.44	0.284	0.30	0.27
Hg	<0.004	<0.004	<0.004	<0.02	0.02	<0.016
Pb	0.036	0.048	<0.004	0.16	0.21	0.093
Se	0.16	0.16	<0.02	0.24	0.24	0.45
F^- (mg/g)	0.005	0.005	†	†	†	0.010
Cl^- (mg/g)	0.02	0.03	†	†	†	0.048
SO_4^{2-} (mg/g)	3.8	4.9	2.0	17	25.6	2.6

[*] Unless otherwise indicated, concentration given in µg/g of dry waste.

[†] Acetate interference.

[‡] Leached in duplicate.

TABLE 4

Number of Times Each Leachate Test Gave the Highest Concentration of an Inorganic Contaminant (Only for Wastes Extracted by All Four Leachate Methods)

Contaminant	ASTM-A	ASTM-B	EP	CAE	Total Comparisons
Ca		3	1		4
Ag	1			1	2
As	1	2		1	1
Ba	1				1
Cd		3			3
Cr		3			3
Hg		1	2		3
Pb	1		2		3
Se	1	1		2	4
SO_4^{2-}	2	2			4
Totals	7	15	5	4	31*
% of Total Comparisons	23	48	16	13	

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*For some leachates, the concentration is below the detection limit and no comparison is made.

TABLE 5

Number of Times Each Leachate Test Gave the Largest Quantity (mass/g of Sample) of an Inorganic Contaminant (Only for Wastes Extracted by All Four Leachate Methods)

Contaminant	ASTM-A	ASTM-B	EP	CAE	Total Comparisons
Ca			4		4
Ag				2	2
As			1	3	4
Ba			2	1	3
Cd		3			3
Cr		1	2		3
Hg			2		2
Pb			3		3
Se				4	4
SO_4^{2-}			3	1	4
Totals	0	4	17	11	32*
% of Total Comparisons	0	13	53	34	

*Comparisons are not made when "less than" values are reported for each leachate method.

It is evident from Table 4 that for most of the inorganic constituents in the leachates, the ASTM methods gave the highest concentration. The ASTM methods account for the highest leachate concentration 71% of the time. This fact can be attributed to the low liquid to solid ratio (4:1), and the large amount of material (350 g) used for the ASTM methods. The ASTM-B method yielded the highest leachate concentrations for many of the trace metals, and probably indicates the ability of the acetate buffer solution to effectively solubilize these cations.

The leachate concentration produced by the ASTM methods gives an estimation of the maximum concentration similar to Ham's Procedure C, using his synthetic leachate. (6) With Ham's Procedure C, the extraction was done three times, with the extracted waste being replaced with fresh material after each elution. The same leaching solution was used throughout the three tests.

Based on the results in Table 5, some leachate procedures exhibit an elemental selectivity. More cadmium is extracted by the ASTM-B method than by the other procedures. This trend is also observed for the extraction of selenium, arsenic, and silver by the CAE method. The EP method extracts the largest quantity of materials, especially the major components, Ca^{2+} and SO_4^{2-}. This is probably due to the higher liquid to solid ratio and the EP's more aggressive agitation method. However, the more vigorous stirring in the EP, could cause the particles to break up and expose new surfaces to the leaching medium. The results, then, might be artificially higher for the EP, and unrealistic in predicting the environmental impact of the waste disposal.

It is difficult to explain some of the elemental selectivity indicated above; more samples need to be tested before being certain that these trends do exist. However, some patterns are apparent and are supported by previous studies. For example, cadmium is preferentially extracted by the leachate solution which has the lowest pH. In most cases, this is the leachate generated by the ASTM-B method. Cadmium, which exists as a cationic species in fly ash, has been shown to be leached more readily in acidic solutions.(3)

Arsenic and chromium are also known to concentrate on the surface of fly ash particulate. The study on trace metal solubility in coal fly ash (3) demonstrated that arsenic and chromium could be solubilized in acidic media, but they were sparingly soluble in H_2O. This information is reflected in the data for the fly ash and hopper ash samples. When the quantity of arsenic and chromium leached by the ASTM-A method is compared with the other three methods, more arsenic and chromium have been extracted in the acidic solutions. When arsenic and chromium exceeded the RCRA threshold values, it was only in the fly ash and hopper ash wastes and only for the leaching tests which used acidic solutions for extraction (Table 6).

TABLE 6

Wastes Classified as Toxic by RCRA Criteria

Waste	Procedure	Element(s) Exceeding Threshold Value
Oil Shale	ASTM-A	Se
	ASTM-B	None*
	EP	None
	CAE	None
Fluidized Bed Combustion Waste	ASTM-A	None
	ASTM-B	None
	EP	None
	CAE	None
Bituminous Coal Fly Ash No. 1	ASTM-A	Se
	ASTM-B	As, Se
	EP	As, Se
	CAE	NR†
Bituminous Coal Fly Ash No. 2	ASTM-A	NR
	ASTM-B	As, Cr, Se
	EP	NR
	CAE	As, Cr
Bituminous Coal Boiler Slag	ASTM-A	None
	ASTM-B	None
	EP	NR
	CAE	None
Lignitic Coal Scrubber Sludge	ASTM-A	Se
	ASTM-B	Se
	EP	Se
	CAE	Se

TABLE 6 (Cont)

Wastes Classified as Toxic by RCRA Criteria

Hopper Ash	ASTM-A	Se
	ASTM-B	Cr, Se
	EP	Se
	CAE	Se

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[*] None - no elements exceeded the threshold value.

[†] NR - Not Run.

Based on the RCRA criteria, five of the energy wastes would be classified as hazardous by at least one of the leachate methods, and the findings are summarized in Table 6. The hazardous leachates were extracts of oil shale, bituminous coal fly ash, scrubber sludge, and hopper ash. Hazardous leachates for the scrubber sludge, and hopper ash were produced by all four methods. In most cases, the concentration of selenium exceeded the maximum acceptable concentration of 10 times the National Interim Primary Drinking Water Standards (0.1 mg/L for Se). In the ASTM interlaboratory program to evaluate the EP and ASTM methods, (1) selenium levels were often in excess of the proposed EPA limits.

The high solution concentrations cited for the ASTM methods in Table 4 are reflected in the number of hazardous leachates produced by the ASTM methods. Four toxic leachates were produced by each of the ASTM methods, while the EP and CAE methods each yielded 3 hazardous extracts.

It is also interesting to note that, regardless of the method used for extraction, selenium often exceeded the RCRA threshold value. In a study of the solubility of trace elements in coal fly ash, (3) it was determined that acidic, neutral, and basic solutions could solubilize selenium from fly ash. A 1 M HNO_3 solution was the most efficient for extracting the selenium, while the H_2O and NH_4OH extracts were comparable in the amounts leached from the fly ash, but were much lower than the acidic solution. The anionic character of selenium in the fly ash could account for its partial solubility in the H_2O extraction (ASTM-A method). Selenium is probably present as the selenate ion (SeO_4^{2-}) which is leached more readily in H_2O than a cationic species such as cadmium.

The percentages of the metals leached from the hopper ash have been calculated in Table 7. Analysis of the hopper ash was conducted by the GCA Analytical Laboratory. After total digestion of the hopper ash, the metals were measured by flame AA. The results are reported in µg/g, with the exception of calcium, which is listed as percent CaO. No percentage is reported for results which were below the detection limits for the AA analyses.

Many of the results indicate that less than 1% of the metal was leached from the waste. Chromium was extracted in a greater percentage than the other metals. The percentage of chromium extracted is especially high for the fly ash samples leached by the ASTM-B and CAE methods. This reemphasizes the availability of chromium on the surface of fly ash and its solubility in acidic media. It appears that most of the other trace metals may be bound to the sample matrix in a manner which makes them unavailable for leaching. Another possibility is that the compound forms of the trace metals are not solubilized by the leaching media.

TABLE 7

Percentage Leached from the Hopper Ash

Concentration of Metal* in Waste			ASTM-A	ASTM-B	EP	CAE
Ca (% as CaO)	=	22.9	1.2	5.4	27	2.0
Ag	=	–	–	–	–	–
As	=	32.9	0.3	0.8	1.2	5.2
Ba	=	4800	0.2	0.01	0.08	0.2
Cd	=	6.06		0.4	0.08	0.1
Cr	=	134	0.4	1.6	3.4	2.4
Hg	=	0.46	–	–	–	–
Pb	=	97	0.03	–	0.3	0.1
Se	=	–	–	–	–	–

---

*Results of AA analysis given in μg/g unless otherwise indicated.

Precision of Leachate Methods

Another means of evaluating the four leachate procedures is to compare the precision of replicate extractions. The relative standard deviation (RSD) has been calculated for the replicate extractions of hopper ash and is presented in Table 8. A comparison of RSD indicates that the precision for the ASTM methods is almost the same, with the ASTM-A method having slightly better reproducibility. The Carbonic Acid Extraction (CAE) is a close third in precision behind the ASTM methods. The Extraction Procedure had the worst reproducibility for the hopper ash extractions. A comparison of the precision for other wastes also indicated that the RSD for the EP was below that of the other methods.

The precision of the methods tested in this study can be compared with the results of previous investigators. One study found that the intralaboratory precision for the EP was quite good with chromium and lead having relative standard deviations of less than 5%. (8) Another investigation into the interlaboratory precision of the EP demonstrated that the relative standard error ranged from ± 9.2% for arsenic in refinery sludge extract to ± 63% for arsenic in fly ash leachate.(2) For the ASTM program, the leachate results from 18 laboratories indicated extreme variability for the EP and ASTM methods. (1) One conclusion drawn from the ASTM data was that the three leachate tests exhibited no consistent difference in precision.

A comprehensive study was undertaken by the Electric Power Research Institute (EPRI) to evaluate the reproducibility of the proposed RCRA Extraction Procedure. (4) It is apparent that most of the variation in the results for the metals in the leachates is due to the analyses and not extraction variability. Most of the variation in the results of arsenic, barium, cadmium, and lead analyses by graphite furnace and barium and selenium by flame AA could be attributed to inter-lab analytical variability. This conclusion would account for the poor precision cited in the ASTM interlaboratory study.

Compatibility with Analytical Methods

The inherent toxicity of the EP leachate to some bioassay tests has been documented by a study at Oak Ridge National Laboratory. (5) Since the acetate ion was the source of the bioassay problems, the ASTM-B method would be expected to exhibit the same problems.

The acetic acid matrices of the EP and ASTM-B methods also caused some analytical problems. These leachate media caused rapid deterioration of the graphite tubes. Standard solutions had to be injected more frequently to monitor the condition of the graphite tubes, resulting in increased analytical time and, with more frequent tube replacement, increased expense.

TABLE 8

Calculation of Relative Standard Deviation from Results of Hopper Ash Extractions

	Relative Standard Deviation (%)			
	ASTM-A	ASTM-B	EP	CAE
Ca	7.9	2.6	0	7
Ag	–	–	–	–
As	5.5	8.5	39	14
Ba	12	4.5	11	1.1
Cd	–	16	92	29
Cr	4.9	5.7	42	7.8
Hg	–	–	–	–
Pb	4.7	–	14	0
Fe	13	14	18	19
F^-	1.5	†	†	40
$C\ell^-$	0	†	†	4.0
SO_4^{2-}	15	17	20	9.1

†Acetete interference

It is also possible that the acetic acid matrix caused some of the variation in the results observed for the EP. The injection of strong acidic solutions into the graphite furnace can degrade the analytical precision. The strong acidic solutions "wet" the inner surface of the graphite tube, and cause variable distribution of the injected sample. This variable distribution will reduce the precision of replicate injections. However, the reduced analytical precision for the EP does not sufficiently account for the differences in precision among the EP and the other three leachate tests.

The acetate ion in the ASTM-B and EP solutions interfered with the determination of fluoride and chloride in the IC analyses. The retention time of the acetate ion is comparable to the retention times of the fluoride and chloride ions and its presence in large excess masked the determination of the fluoride and chloride concentrations.

CONCLUSIONS AND RECOMMENDATIONS

When the four leachate procedures are evaluated in terms of the general applicability, reproducibility, methods compatibility, and leaching power, the ASTM-A and Carbonic Acid Extraction would be preferred for a standard leachate test. In terms of leaching power, the CAE extracts a greater quantity of inorganic contaminants than ASTM Method A. However, the reproducibility of ASTM-A is a little better than the CAE. No analytical problems were encountered with either method and both procedures could adequately handle the wastes tested in this program. The leachates from the CAE would have to be subjected to the bioassay tests to determine the compatibility of the CAE with the health and ecological tests.

The major criticism of all the proposed methods is the lack of sufficient detail in the procedures. A standard leachate procedure should be explicitly defined to avoid interpretation by the analyst and, consequently increase the reliability of interlaboratory results.

The objectives of the leachate test should be of primary concern. Is the test attempting to simulate acid rain conditions, or degradation at a disposal site? Once the objectives of the test have been established, then the procedure to achieve these goals can be designed. The separation of complex, multi-phase industrial wastes prior to leaching has not been covered by any of the methods. A separation scheme similar to that suggested by Ham (6) could be adopted. Sample preparation, or whether to use the waste in its disposed form, is another question which needs to be addressed. The portion of the leachate methods most susceptible to interpretation by the analyst is the agitation method. If the shaking apparatus and agitation rate is rigidly defined, much of the variability in the leachate results would be eliminated. Finally, the means for preserving the sample for subsequent analyses must be included in the standard leachate test.

ACKNOWLEDGEMENTS

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POSSIBLE EFFECTS OF COLLECTION METHODS AND SAMPLE PREPARATION ON LEVEL 1 HEALTH EFFECTS TESTING OF COMPLEX MIXTURES

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ABSTRACT

Level 1 Environmental Assessment is oriented toward the development of a data base that will permit a relative ranking of industrial streams with respect to their potential biohazard. The combination of both chemical and biological evaluation of collected samples in the assessment increases the level of data integration and coordination, but reduces the chance that a potentially hazardous stream will go undetected. During the initial phases of the IERL Environmental Assessment Program, considerable time was given to methods of sample collection, preparation and analysis for chemistry assessment (IERL-RTP Procedures Manual: Level 1 Environmental Assessment (Second Edition) EPA-600/7-78-201, October, 1978). The recent introduction of bioassays requires a similar appraisal of the functions of sample collection, storage and pretest handling as they relate to the specific health effects and ecological tests proposed for Level 1 biological assessment.

Because of the need to rank streams according to potential biohazard, the initial approach taken by IERL was to evaluate samples in the specific bioassays in a state as similar as possible to that found at the time of sampling. However, recent results reported in the scientific literature on analysis of complex environmental mixtures have shown that pretest processing of samples (concentration of liquids, extraction of particular) often results in enhanced biological activity (6,7,9,10,11).

The purpose of this discussion is to review several of the sampling and pretest procedures and to illustrate how the application of these techniques to Level 1 Environmental Assessment will affect the test responses and ultimately the goals of this program.

GOALS OF LEVEL 1 ENVIRONMENTAL ASSESSMENT

The fundamental goals of IERL Level 1 Environmental Assessment are to reliably detect potentially hazardous emissions from stationery source sites and to rank the streams in order of their need for control technology application (Figure 1). These goals must be attained in a cost-effective manner so that many different emission sources can be sampled and evaluated and repetitive samplings from the same source can be made.

The basic approach proposed for Environmental Assessment has been an evaluation of emissions for the following five parameters:

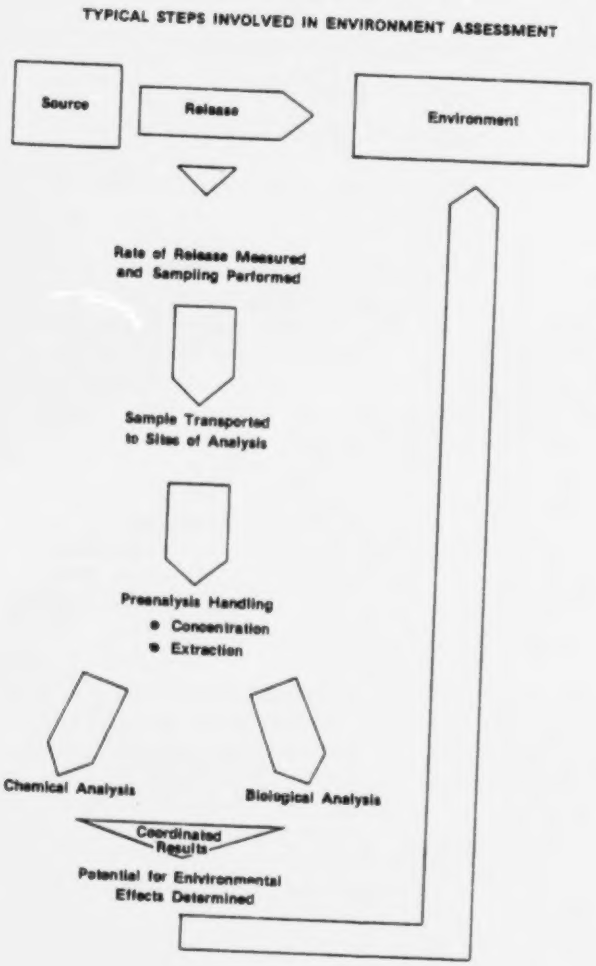
TYPICAL STEPS INVOLVED IN ENVIRONMENT ASSESSMENT
Source
Release
Environment
Rate of Release Measured and Sampling Performed
Sample Transported to Sites of Analysis
Preanalysis Handling
• Concentration
• Extraction
Chemical Analysis
Biological Analysis
Coordinated Results
Potential for Enivironmental Effects Determined

FIGURE 1 An overview of the steps involved in Level 1 Environmental Assessment.

1. The rate of release of the emission into the environment.

2. The distribution between physical states of the released emission products in the environment.

3. Chemical composition of the emission.

4. Detection of potential impact of the emission on specific health effects.

5. Detection of potential impact of the emission on the ecosystem.

This document will focus on parameters 4 and 5 with less emphasis placed on items 2 and 3.

The initial Level 1 methods manual for biological testing contained protocols for five health effects tests and eight biological tests. The types of data generated from this array of tests were quite different and not amenable to interpretation by non-biological scientists. For their optimum use, a method of developing uniform data sets was needed. A review of Level 1 bioassays and data generated from several pilot studies resulted in recommendations concerning uniform methods for data analysis and formatting (8). It was also recognized in this review process that elimination and/or substitution of test procedures would benefit the Level 1 assessment. Table 1 identifies the current status of recommendations for Level 1 bioassays; however, there are no current procedures approved for E5. Test substitutions or protocol modifications are under consideration for some of the other tests as well. These will be given in more detail in the second edition of the Level 1 Manual for biological testing.

A summary of three pilot studies using a battery of Level 1 bioassays employing this data-formatting system have been reported (8). Employing the recently proposed data-formulating procedures, the results from the pilot studies are reported as high (H), moderate (M), low (L), or nondetectable (ND) in a summary table (Figure 2) according to the scheme given in Table 2. One feature of the pilot studies was that the results from the studies on Coal Gasification Fluidized Bed Combustion and Textile Plan Liquid Effluents were evaluated with very little pretest sample handling or sample history associated with the samples used in the bioassays. For example, water samples were not concentrated, leachates were not concentrated and particulate samples were not extracted with organic solvents. Some filter or sorbent collectors were extracted with solvents or sonicated with solvents, but in large part, most of the samples were placed in the bioassays as received by the testing facilities.

Comparative analysis of the chemistry and biology of these pilot programs has been shown to be generally complementary between the severity assessment based on chemicals detected and biological responses (1). In a few instances toxicity was not predicted by the chemical analysis.

What effect pretest processing might have on such data is important since the Level 1 procedures are being used more frequently. Should samples be

TABLE 1
LEVEL 1 BIOASSAYS

Health Effects Bioassays

H1. Bacteria Mutagenesis Assay
H2. CHO Clonal Toxicity Assay
H3. RAM Assay
H4. *In Vivo* Rodent Toxicity

Ecological Assays

Freshwater Aquatic/Marine Aquatic
E1. Fish
E2. Inertebrate
E3. Algal
Terrestrial
E4. Insect Toxicity
E5. Soil Microorganism Toxicity
E6. Plant Toxicity

Characteristics	H1	H2	H3	H4	E1	E2	E3	E4	E5	E6
1. Test Sample Applications										
· Solid	+	+	+	+	+	+	+	+/-**	-	-
· Liquids	+	+	+	+	+	+	-	+	+	+
· Gases	+/-	-	-	-	-	-	-	+	-	+
· Mixtures (Solid/Liquid) and Slurrys	+	+	+	+/-	+/-	+/-	+/-	+/-	+/-	-
· Filter or Sorbent Extracts	+	+	+	-	-	-	-	+	-	-
2. Test Results Applicable to Chronic Toxicity	+	-	-	+/-	-	-	-	+/-	-	-
3. Bioassay Includes Life Cycle Analysis	-	-	-	-	-	-	-	+	-	-
4. Quality Control Procedures Established	+	+	+	+	-	-	-	+	-	-
5. Test Method in Developmental State	-	-	-	-*	-	-	+/-	+/-	+***	+

*Protocol changed from rat to mouse

**Depends on solubility of materials

***No test currently proposed for Level 1.

TABLE 2

DEFINITION OF EFFECTIVENESS CATEGORIES

ASSAY	ACTIVITY MEASURED	UNITS	MAD	RANGE OF CONCENTRATION OR DOSAGE HIGH	MODERATE	LOW	NOT DETECTABLE
Ames Test	Mutagenesis*	mg/plate	5	<0.05	0.05-0.5	0.5-5	ND at >5
		μl/plate	50	<0.5	0.5-5.0	5-50	ND at >50
RAM/WI-38 and CHO TOXICITY	Lethality EC50	mg/ml	1	<0.01	0.01-0.1	0.1-1	ND at >1
		μl/ml	600	<6.0	6.0-60	60-600	ND at >600
		μl/ml	20 **				ND at >20
RODENT TOXICITY	Lethality LD50	gm/kg	10	<0.1	0.1 -1	1 -10	ND at >10
Aquatic Tests							
Algae	Growth Inhibition EC50	gm/liter %	1 100	<0.01 <20	0.01-0.1 20-75	0.1-1 75-100	ND at >1 ND at >100
Fish	Lethality LC50	gm/liter %	1 100	<0.01 <20	0.01-0.1 20-75	0.1-1 75-100	ND at >1 ND at >100
Invertebrate	Lethality LC50	gm/liter %	1 100	<0.01 <20	0.01-0.1 20-75	0.1-1 75-100	ND at >1 ND at >100

Abbreviations:
MAD = Maximum Applicable Dose (Technical Limitations)
LD50 = Calculated Dosage Expected to Kill 50% of Population
LC 50 = Calculated Concentration Expected to Kill 50% of Population
EC50 = Calculated Concentration Expected to Produce Effect in 50% of Population
ND = Not Detectable

*Negative response at 5 mg/plate or at level of toxicity is given as ND. Positive response requires calculation of minimum effective concentration (MEC) to produce a positive mutagenic response. H, M and L designations are made from MEC values of positive agents.

**Volumes used for solvent exchange samples (This maximum keeps DMSO below level of toxicity).

BIOASSAY SUMMARY

Technical Directive or Project No. ____________________

Contract No. ____________________

						Fresh Water			Marine					
Sample Identification	**Salmonella**	**Ram Cytotoxicity**	**WI-38 Cytotoxicity**	**CHO Cytotoxicity**	**Rodent Toxicity**	**Fish**	**Invertebrate**	**Algal**	**Fish**	**Invertebrate**	**Algal**	**Plant Stress Ethylene**	**Soil Test**	**Notes**

ND = No Detectable Toxicity
L = Low Toxicity
M = Moderate Toxicity
H = High Toxicity

FIGURE 2 Data from all tests of one stream can be translated into toxicity definitions and compared.

used directly from the sampling process or should an effort be made to reduce each sample to its active constituents or to concentrate dilute samples?

In addition to pretest processing, other aspects of Level 1 assessment have been identified as important components in the final data analysis and interpretation. These aspects are listed below:

1. Sampling Methods -- Selection of representative sample.
2. Storage -- Important in maintaining proper composition and prevention of degradation.
3. Shipping (Transport) -- Same as 2.
4. Pretest Handling -- Alter chemical composition, physical state or preferential extraction and concentration of potential toxicants and mutagens.

Items 1, 2 and 3 can be grouped into a single parameter called sample history (i.e., documentation of all handling of the samples up to receipt at the testing laboratory). Item 4 encompasses several pretest sample processing techniques carried out at the testing facilities (see Table 3).

Another factor not listed above but of real significance to the final assessment is one of environmental fate of the released emission and in particular, bioaccumulation. Significant bioaccumulation of weak or moderately toxic substances in emissions might have considerable environmental impact. These considerations are examined in detail in the following sections.

SAMPLE HISTORY

The reasons for emphasis on sample history relate to the final interpretations of the test results and the ranking of emission sources with respect to potential hazard.

The emission rate profoundly influences environmental assessment since large volume emissions (e.g. fly ash from power plants) provide an opportunity for significant human and ecosystem exposure (3,7). An emission with extremely low volume and rate of release may represent a negligible health or ecological hazard even if it is highly toxic.

For example, if one identifies a potential hazard* as a function of release rate (volume/unit time), its toxicity and its environmental fate, a quantified assessment may be possible. Thus, if any single component is very large, the potential hazard could be significant. Attempts should be made to document the history of each sample put into the Level 1 bioassay program.

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* Potential hazard is not synonymous with risk assessment. The types of bioassays used in Level 1 are able to detect potential but are not capable of estimating risk either qualitatively or quantitatively.

TABLE 3
LEVEL 1 BIOASSAYS

Pretest Handling	Method Employed	Possible Effect
Particulate Extraction	· Organic solvent/sonication · Soxhlet/organic solvent	Preferential release of toxic and mutagenic organic materials from bound state. If these organics are not released under normal environmental or physiological conditions they can skew ranking scheme.
Liquid Concentration	Flow through XAD-2 resin column followed by soxhlet extraction from resin and solvent exchange	Concentrates organics and permits inorganics to pass through. Some of the inorganics may be important toxicants. Again, preferential concentration of organics might increase detection of mutagens and skew ranking scheme. Concentration of chemicals may introduce artifacts by altering chemical: chemical dynamics not encountered in dilute solutions.
Extraction from Collection Filter	Sonication in organic solvent	Preferential release of toxic and mutagenic agents preferentially attached to filter material. Degredation and alteration of chemicals is known to occur when bound to filter. Sonication of filter may release small particles that would be toxic in RAM assay.
Solvent Exchange and Solubilization	Addition of sample to methylene chloride, acetone, benzene, DMSO, etc.	Preferential release of soluble compounds to the target organism. Nonrepresentative composition of original sample by extraction of organics.
Particulate Sizing and Grinding	· Filter Collection · Grinding and sizing with mill and wire screen	Development of a physical state more amenable to cellular phagocytosis or animal absorption. This could skew ranking by producing abnormally high levels of toxicity.

Currently only the toxicity factor is employed in the Bioassay Summary Table (Figure 2). This factor is not tied back to the type of emission release rate or to the volume of emission sampled.

PRETEST SAMPLE PROCESSING

Several laboratories in the U.S. (particularly at the EPA IERL in RTP and the Oak Ridge National Laboratory) have initiated programs to analyze complex mixtures for mutagenic activity (2,4). The Ames *Salmonella* assay is typically employed in these programs and is conducted on fractions of the complex mixtures as a means to direct further fractionation tests in the pursuit of biologically active components (pure substances).

The general procedures are to: (a) collect the desired sample (particulate, gas or liquid) on a filter or solid sorbent, (b) extract the organics from the filter or sorbent by sonication or Soxhlet methods into an organic solvent, (c) exchange solvents (to DMSO) or evaporate to dryness and resuspend in DMSO and (d) conduct the bioassay on the concentrated extract. Chemical fractionation and further testing may eventually lead to specific associations between chemicals or chemical classes with biological activity (4). Table 4 describes some of the pretest sample processing techniques currently used and demonstrates how these methods could affect the interpretation of Level 1 bioassay data using conventional data evaluation techniques.

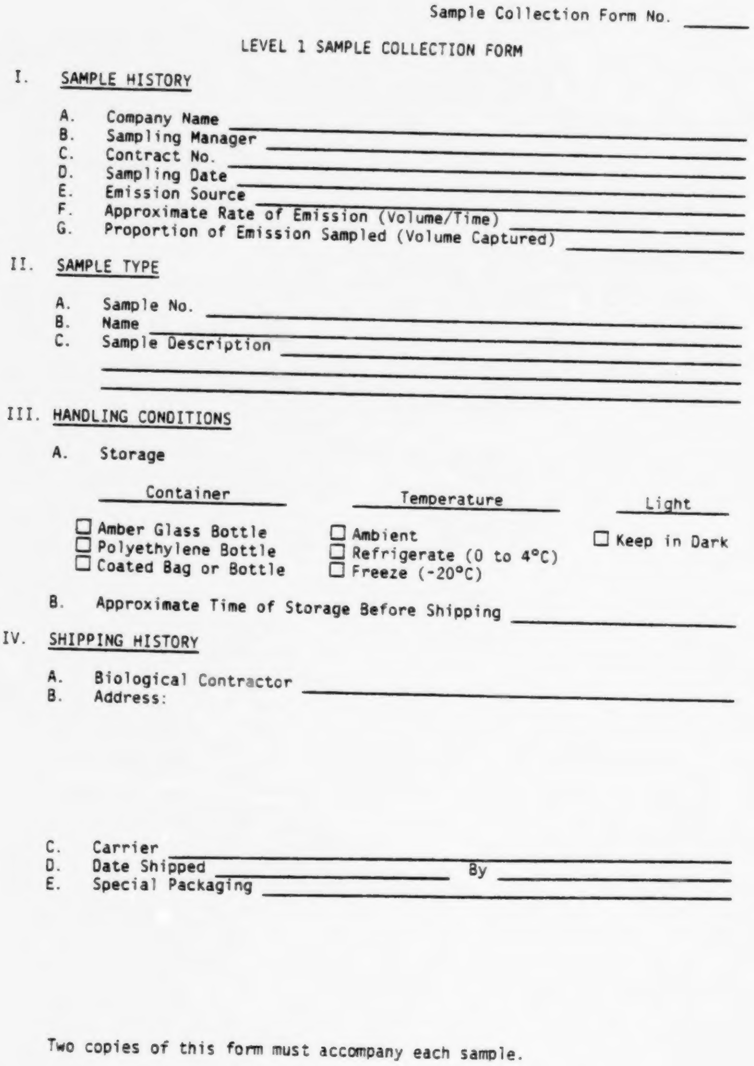
Level 1 toxicity assessments listed in the Bioassay Summary Table (Figure 2) include several types of biological systems and phylogenetic levels. If a significant amount of pretest processing is anticipated, the resultant extracts and concentrates should be evaluated in all the Level 1 tests and not just the Ames or one or two selected tests. If unprocessed samples are used in the remaining tests on the site emission, the balance of test results may be altered.

Pretest processing should be included in evaluation of all Level 1 test results, otherwise the data balance will be upset and the ranking of the test site might be biased by an abnormally toxic response obtained from an extract or concentrate in a single assay. This could erroneously exaggerate the potential hazard.

The previous precaution is not meant to preclude pretest processing, but as with the sample history factor (release rate) described previously, the pretest sample processing must in some way be factored into the final assignment of a toxicity value and its contribution to the potential hazard. The mechanism to accomplish this need appears to be rather complex. In the simplest approach, one might want to divide the actual test response by a concentration factor to normalize the effect back to the pre-processing state.

RECOMMENDATIONS

1. Documentation of sample collection, storage, shipping and pretest processing should be available for all test samples. Examples of forms for this purpose are shown in Figures 3 and 4.



Sample Collection Form No. ______

LEVEL 1 SAMPLE COLLECTION FORM

I. SAMPLE HISTORY

A. Company Name ____________________
B. Sampling Manager ____________________
C. Contract No. ____________________
D. Sampling Date ____________________
E. Emission Source ____________________
F. Approximate Rate of Emission (Volume/Time) ____________________
G. Proportion of Emission Sampled (Volume Captured) ____________________

II. SAMPLE TYPE

A. Sample No. ____________________
B. Name ____________________
C. Sample Description ____________________

III. HANDLING CONDITIONS

A. Storage

Container	Temperature	Light
☐ Amber Glass Bottle ☐ Polyethylene Bottle ☐ Coated Bag or Bottle	☐ Ambient ☐ Refrigerate (0 to 4°C) ☐ Freeze (-20°C)	☐ Keep in Dark

B. Approximate Time of Storage Before Shipping ____________________

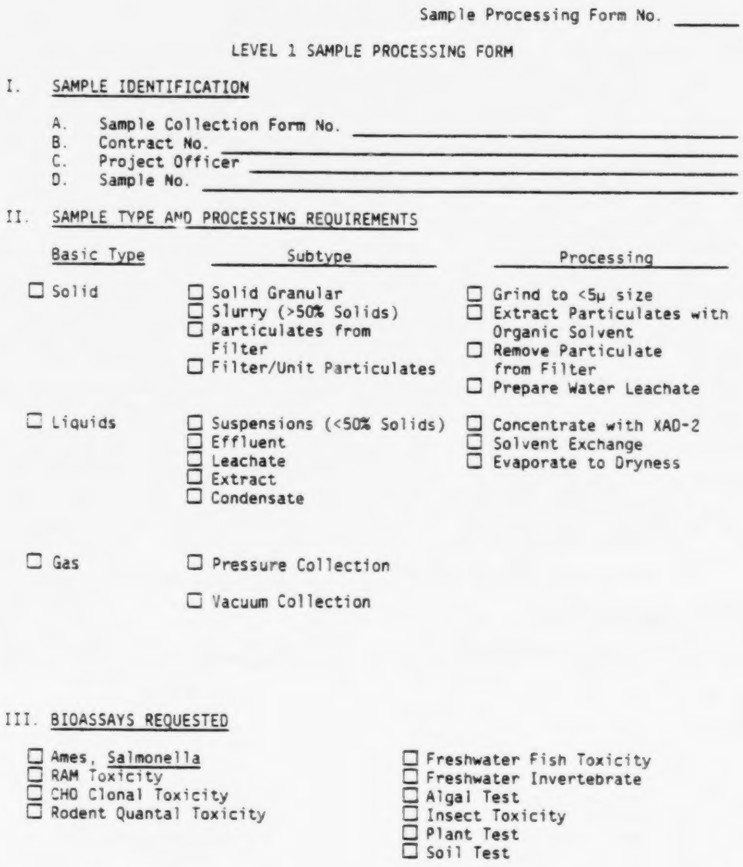
IV. SHIPPING HISTORY

A. Biological Contractor ____________________
B. Address:

C. Carrier ____________________
D. Date Shipped ____________________ By ____________________
E. Special Packaging ____________________

Two copies of this form must accompany each sample.

FIGURE 3 A possible form on which sample history can be collected to establish the conditions of collection, storage, and shipping.



Sample Processing Form No. ______

LEVEL 1 SAMPLE PROCESSING FORM

I. SAMPLE IDENTIFICATION

A. Sample Collection Form No. ____________________
B. Contract No. ____________________
C. Project Officer ____________________
D. Sample No. ____________________

II. SAMPLE TYPE AND PROCESSING REQUIREMENTS

Basic Type	Subtype	Processing
☐ Solid	☐ Solid Granular ☐ Slurry (>50% Solids) ☐ Particulates from Filter ☐ Filter/Unit Particulates	☐ Grind to <5μ size ☐ Extract Particulates with Organic Solvent ☐ Remove Particulate from Filter ☐ Prepare Water Leachate
☐ Liquids	☐ Suspensions (<50% Solids) ☐ Effluent ☐ Leachate ☐ Extract ☐ Condensate	☐ Concentrate with XAD-2 ☐ Solvent Exchange ☐ Evaporate to Dryness
☐ Gas	☐ Pressure Collection ☐ Vacuum Collection	

III. BIOASSAYS REQUESTED

☐ Ames, *Salmonella*
☐ RAM Toxicity
☐ CHO Clonal Toxicity
☐ Rodent Quantal Toxicity
☐ Freshwater Fish Toxicity
☐ Freshwater Invertebrate
☐ Algal Test
☐ Insect Toxicity
☐ Plant Test
☐ Soil Test

FIGURE 4 A possible form for use in describing the pretest processing and types of bioassays to be used with a sample.

2. Pretest processing should be factored into the final determination for toxicity designations of H, M, L and ND. Specific methods need to be developed to normalize data obtained from samples modified prior to evaluation.

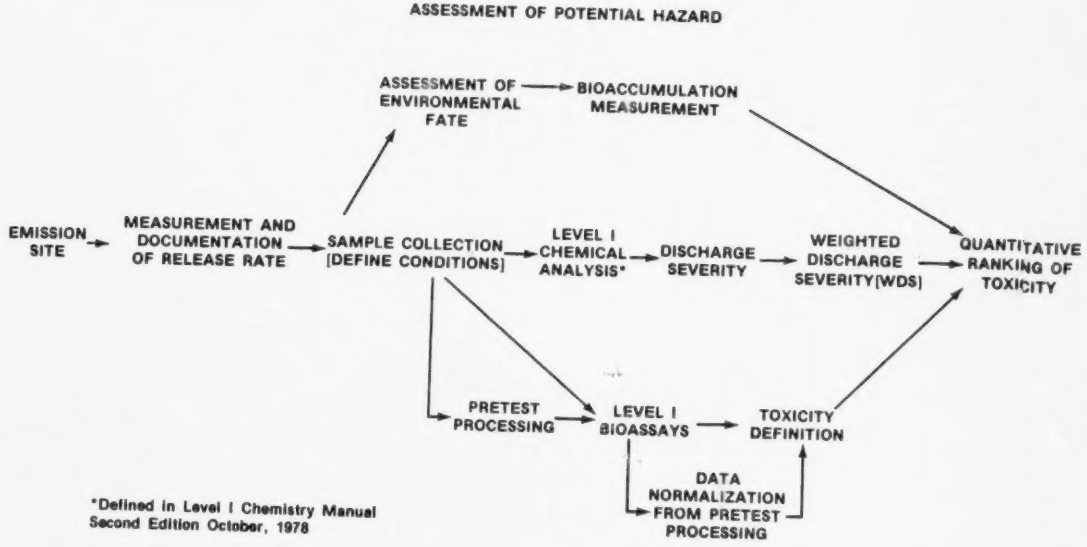
3. Emissions from a given site should be applied to the spectrum of Level 1 bioassays uniformly and not in a manner likely to bias the final interpretation. Data related to environmental fate should be included.

4. Discharge severity (DS) calculations presently used to factor chemical and physical information should be expanded to include biological response and fate. Specific methods need to be developed.

SUMMARY/CONCLUSIONS

Level 1 Environmental Assessment Bioassays should permit an accurate ranking of emissions from stationary site sources with respect to their potential hazard. Moreover, the ranking must ensure that the potential hazard is likely to be derived from the emissions as released into the environment and that pretest processing should be kept to a minimum and applied uniformily across all Level 1 bioassays if performed.

Level 1 Assessment should include the environmental fate and rate of release of the emission along with the chemical and bioassay toxicity determinations. This approach would result in a second level determination called a severity potential hazard. A general scheme proposed to develop this assessment is given in Figure 5. If a potential hazard can be calculated with reasonable accuracy the utility of Level 1 results will be greatly enhanced.


ASSESSMENT OF POTENTIAL HAZARD
ASSESSMENT OF ENVIRONMENTAL FATE
BIOACCUMULATION MEASUREMENT
EMISSION SITE
MEASUREMENT AND DOCUMENTATION OF RELEASE RATE
SAMPLE COLLECTION [DEFINE CONDITIONS]
LEVEL I CHEMICAL ANALYSIS*
DISCHARGE SEVERITY
WEIGHTED DISCHARGE SEVERITY[WDS]
QUANTITATIVE RANKING OF TOXICITY
PRETEST PROCESSING
LEVEL I BIOASSAYS
TOXICITY DEFINITION
DATA NORMALIZATION FROM PRETEST PROCESSING

*Defined in Level I Chemistry Manual
Second Edition October, 1978

Figure 5 PROPOSED SCHEME FOR A SECOND STAGE EVALUATION OF LEVEL 1 RESULTS.

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THE DYNAMIC INTERACTION BETWEEN VAPOR PHASE AND PARTICULATE MATERIALS

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COAL FLY ASH AS A MODEL COMPLEX MIXTURE FOR SHORT-TERM BIOASSAY

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ABSTRACT

National energy policy has designated coal as the major energy source for production of electricity throughout the remainder of this century. Associated with the combustion of coal is the generation of vast quantities of primary mineral matter, fly ash. This report describes the application of bioassays including bacterial mutagenesis, mammalian lung cell function and mammalian hematopoietic cell maturation to the study of the biologically significant physical and chemical properties of coal fly ash. The effects of sample collection, particle size, solvent extraction, temperature treatment and irradiation are discussed.

INTRODUCTION

Combustion of coal for electric power generation has increased markedly throughout the past decade and is expected to continue to increase throughout the remainder of this century. Associated with coal combustion is the production of a variety of biologically active, inorganic and organic compounds. Of major concern is the release of oxides of carbon, nitrogen, and sulfur, biologically active trace elements, siliceous primary particulate matter and organic compounds. Coal fly ash represents the bulk of the primary particulate matter produced during coal combustion. Interaction of the fly ash particles with inorganic and organic compounds formed during the combustion process results in the formation of a unique complex mixture that may serve as a useful model for other such mixtures resulting from interaction of a relatively inert carrier particle with biologically active metals and organic compounds. In a similar manner, the bulk of atmospheric particulate matter may serve as a matrix for subsequent interaction of airborne vapors and condensable gases.

SAMPLE COLLECTION

The fly ash samples described in the following studies were collected from a western U.S. power plant burning low sulfur (0.5%), high ash (20%) coal (29). Unless otherwise indicated, the samples were collected and size fractionated *in situ* downstream of the plant's electrostatic precipitator (ESP). On occasion, studies of ESP-collected ash were also performed. Four fractions of stack-collected material were obtained with volume median diameters (VMD) of 20, 6.3, 3.2 and 2.2 μm, respectively, and geometric standard deviations of approximately 1.8. Size classification was performed utilizing a specially designed thermostated (95°C) system containing two cyclones in series followed by centripeter with 25 parallel jets (29). Cyclone-separated material was deposited in a collection hopper while centripeter particles were collected on fabric filters and removed for hopper deposition by cleaning with reverse air jets operating at one-minute intervals. Thus, in contrast to standard filter collection techniques, collected fly ash samples were not continually exposed to the reactive gases in the flue stream. Such an exposure may lead to changes in the chemical composition and biological activity of collected polynuclear aromatic hydrocarbons (4).

It should also be pointed out that our samples were collected continuously over a 30-day period. Thus, alterations in coal composition or combustion conditions and other parameters of plant operation that may affect chemical composition should be reflected in these samples. In this regard, Kubitschek and Kirchner (28) have demonstrated the important effects on mutagenic activity of combustion conditions during start-up and shut-down of a bench-model, fluidized-bed combustion system.

PHYSICAL AND CHEMICAL CHARACTERIZATION

Microscopic Studies

In previous studies (8,10,16), we have described the physical and morphological properties of coal fly ash and generally find that coal fly ash is an extremely heterogeneous, complex mixture with a variety of morphological forms. Variation in morphology generally represents both matrix composition and exposure conditions during combustion. Upon heating, aluminosilicate inclusions in the coal initially become rounded, and, through degassing, become vesicular (8). Further heating will result in sphere formation, either solid sphere, hollow sphere (cenosphere), or sphere-within-sphere structures (plerosphere). Crystal formation is a somewhat later event in morphogenesis with internal crystals or quench crystals forming rapidly during the phase transition from liquid to solid (10). Such quench crystals have been identified as mullite by Gibbon (20). While internal (quench) crystal formation occurs in a time frame of milliseconds, surface crystal formation appears to be a much slower process taking days or months. Such surface crystal formation appears to be the result of sulfuric acid interaction with metals found on fly ash surfaces.

Previous efforts on the analysis of individual fly ash particles demonstrated extreme matrix heterogeneity between morphologically similar particles (35). We have more recently completed a detailed comparison of light-microscopic morphology and individual-particle, elemental composition using SEM X-ray analysis. The utilization of comparative, light and electron microscopy provides a powerful tool for the assessment of physical and chemical properties of coal fly ash (15). Our findings have demonstrated the presence of relatively pure mineral phases although, certainly, the bulk of the ash is amorphous and appears to have the composition of the clay minerals generally found to be associated with coal. Pure alpha-quartz, calcium phosphate, titanium dioxide, calcium oxide, iron oxide, and alumina have been observed (25). The degree of pigmentation of spherical particles has clearly been shown to be the result of varying iron content within the particles. Statistical cluster analysis has confirmed the utility of the light microscopic morphological classifications (15). In particular, we have shown that the morphological classifications defined by light microscopy generally possess a relatively high degree of homogeneity with regard to elemental compositions of individual particles within the specific morphological classes. It should be pointed out, however, that the most morphologically amorphous particles tended to have the greatest diversity in elemental composition.

We have also recently completed studies on the crystalline phases within coal fly ash using X-ray diffraction analysis (24). These studies demonstrated that the predominant crystalline species in coal fly ash are alpha-quartz, mullite, and magnetic iron oxides. The highest concentration of crystalline material was found in the coarsest fly ash fractions with quartz concentrations of 4 percent, mullite of 8 percent, and iron oxide of 0.6 percent. The concentrations of crystalline minerals decreased with decreasing particle size such that alpha-quartz in the finest fly ash fraction was 1.3 percent, mullite 4 percent, and magnetic oxides 0.03 percent. It is hypothesized that the silica is the result of quartz intrusions within the coal itself, whereas mullite and magnetite are formed during the combustion and cooling processes associated with utilization of the coal. It should be pointed out that the mullite is generally associated with a quench crystal phase that occurs during rapid cooling, and hence, is generally encapsulated within the aluminosilicate matrix of the clay mineral. This does not appear to be the case for alpha-quartz or magnetic iron oxides.

As described in our earlier work, surface crystal formation may take place (10,16). This is apparently the result of chemical interaction or formation of sulfuric acid on fly ash surfaces. Leaching of minerals and heavy metals from within the fly ash by the surface associated sulfuric acid may result in crystal formation. Electron microprobe analysis of larger crystals found in fly ash has identified only calcium and sulfur to be present; hence, the majority of crystals appear to be calcium sulfite, either present as gypsum or anhydrite. We postulate, however, that the mechanism of sulfate crystal formation may also provide for the increased biological availability of refractory metal oxides by conversion to the more soluble metal sulfates.

As described by many other investigators, we have found that the concentration of the volatile (at coal combustion temperatures) trace elements or their oxides are highest in the finest fly ash particles (7,34). In this regard, cadmium, zinc, selenium, arsenic, antimony, tungsten, molybdenum, gallium, lead, vanadium, fluorine, and sulfur are most enriched in the finest fly ash particles. However, one also finds that the relatively refractory elements, uranium, chromium, barium, copper, beryllium, and manganese are enhanced in the finest fly ash fractions. While it appears that enhancement of volatile trace elements in fine fly ash particles is due to vapor phase condensation as described by Natusch et al. (33), other mechanisms may also be important in this phenomenon. Filtration studies with neutron-activated coal fly ash indicate that some elements are highly enriched in particles in the size range from 0.2 to 0.4 micrometers (17). In particular, the elements antimony, arsenic, tungsten, uranium, and chromium have a disproportionately high concentration in particles less than 0.4 micrometers. It has been hypothesized that the enhancement in submicron particles is the result of homogeneous nucleation and subsequent coagulation of primary particles or the condensation of reaction products. The presence, however, of relatively high concentrations of biologically active trace elements in the submicron mode may present special problems for particulate abatement technologies.

We have performed further studies to evaluate the relative distribution of elements in fly ash associated with the aluminosilicate matrix compared to those either in separate mineral phases or associated with the particulate surface (23). Preferential dissolution techniques utilizing either hydrochloric acid or hydrofluoric acid indicated that greater than 70 percent of the titanium, sodium, potassium, magnesium, hafnium, thorium, and iron is associated with the aluminosilicate matrix. On the other hand, more than 70 percent of the volatile elements arsenic, selenium, molybdenum, zinc, cadmium, tungsten, vanadium, uranium, and antimony are associated with the particulate surface. It also appears that the majority of the calcium, scandium, strontium, lanthanum, and the rare earth elements is associated with a separate mineral phase, possibly an apatite phase, which has a particle size distribution similar to the aluminosilicate phase. These findings, then, allow for computation of the probable biological availability of trace elements in coal fly ash. In agreement with these observations, we have found relatively high degrees of solubility for molybdenum, calcium, selenium, barium, arsenic, tungsten, zinc, and antimony in coal fly ash treated with a Tris-hydrochloric acid buffer at pH 7.4 (17).

IMMUNOTOXICOLOGY STUDIES

Although many sensitive *in vitro* bioassays exist for mutagenesis, few in vitro assays are available for screening environmental toxicants for their potential effects on host cellular defense factors. The difficulties associated with the development of assays for monitoring immune effects are underscored by the extremely complicated nature of the cellular and humoral factors involved in the host reaction to neoplasia. To this end, we have initiated development of a variety of methodologies for the study of environmental factors affecting cellular immunities. Major effort has been dedicated to the development of assays reflecting inhibition of macrophage function.

In Vitro Studies

Scanning electron microscopic X-ray analytical techniques have been applied to the investigation of particles contained in macrophages (26,35). We have demonstrated that the matrix composition of particles contained within phagocytes may vary dramatically. Furthermore, one may hypothesize that the variation in chemical composition of phagocytized particles may also represent a variation in toxicological potential of such particles (26,27). In this regard, a model has been proposed for the exposure of individual lung cells to the foreign elements contained in fly ash. Segregation of elements in specific particles of fly ash results in a possibility for significantly higher exposure levels within individual cells than that projected from the model of elemental concentrations distributed uniformly among all particles. Furthermore, we have demonstrated the feasibility of comparative microscopic techniques for a correlation of viability of individual cells with the elemental composition of phagocytized particles. Light microscopic analysis of macrophages deposited on SEM finder grids and treated with trypan blue dye indicates the viability of the cells; subsequent electron-microscopic analysis of the same cells provides indication of the elemental composition of the phagocytized particles. Studies are now under way to evaluate the relative toxicity of the various fly ash compositions.

To evaluate whether there is a potential for trace elements contained within fly ash to alter the function or viability of macrophages, we have calculated the elemental content of macrophages after phagocytizing a few fly ash particles (27). In general, our calculations indicate that the concentrations of many biologically active trace element are increased by one, two, or even three orders of magnitude. These calculations, then, indicate that the trace elements in fly ash have a potential for producing cellular damage. Further studies are necessary to evaluate biological availability of trace elements within the fly ash. In this regard, we have found that many trace elements within fly ash are effective inhibitors of either lectin-induced or mixed-lymphocyte-induced lymphocyte blastogenesis (37). Relative to concentrations in coal fly ash, chromium, lead, vanadium, and copper appear to be effective inhibitors of the lymphocyte stimulation assays. It is interesting to note that both lymphocytes and macrophages appear to be extremely sensitive to vanadium.

In vitro exposure of macrophages to particulate matter of similar size distributions has provided a comparison of the relative toxicity of fly ash, silica, and glass beads (13,38). Silica was chosen as a positive control since it is a well-documented, macrophage toxicant. Glass beads or aluminosilicate particles were chosen as a negative control because of apparent inertness. Initial studies of the *in vitro* effects of exposure to these particles have been performed with both rat and mouse pulmonary macrophages (13). Particulate exposure was performed at a 40 to 1 particle-to-cell ratio comparable to concentrations of test particles used in the phagocytic assay (12). Our studies indicate that the phagocytic activity of macrophages increases with time in incubation media (13). Control phagocytosis tends to increase with increased time in culture; the degree and rate of enhancement of phagocytosis is dependent upon the species derivation of macrophages. Rat macrophages tend to show enhancement after two hours in culture whereas murine macrophages tend to exhibit enhancement after two days in culture. Exposure to fly ash resulted in a lag time for increased phagocytic rates. The lag appeared at two and four hours for rat macrophages. Similarly, fly ash produced a lag in phagocytosis compared to controls at one and two days in culture for murine macrophages. Interestingly, although, silica exposure did not produce a lag phase, the final phagocytic capability at seven days was markedly below that of controls. Further studies are necessary to evaluate the significance of such a lag phase as well as to define the nature of the enhanced phagocytosis associated with *in vitro* culture.

We have also developed techniques for evaluation of the proliferative capacity of lavaged cells from the lung (1,2). The clonogenic technique used was adapted from previous techniques utilized for quantitation of bone marrow granulocyte-monocyte progenitors (40). The basic culture system involves the plating of lung-lavaged macrophages into a semi-solid methylcellulose medium. Colonies tend to be rather small and slow to grow compared to the kinetics of hematopoietic cells. Preliminary studies indicate that all particle types, i.e., fly ash, silica or glass beads, may have an effect on the ability of these lavaged cells to divide (38). We have generally found that the response of functional assays is the opposite of the proliferative response. Agents that tend to stimulate phagocytosis appear to result in a decrease in proliferative capacity. Similarly, we have found that agents that inhibit phagocytosis tend to stimulate proliferation. These observations suggest that particle-induced phagocytic functions may preclude differentiation and subsequent division of progenitors.

We have also demonstrated the utility of cloning techniques in the study of the in vitro dose-response characteristics of trace elements on lymphohematopoietic progenitors using semi-solid culture systems (39). Because of the enhanced concentration of zinc and selenium in fine fly ash particles, and because of the known biological activity of these elements, we evaluated the response of murine spleen B-lymphocyte progenitors and bone marrow granulocyte-monocyte progenitors to selenite and zinc exposures. Interestingly for both elements, a significant suppression of cell proliferation from splenic B-lymphocytes was observed at physiological concentrations. This was not the case for the granulocyte-monocyte progenitors. The results demonstrate the feasibility of using lymphohematopoietic cloning techniques as sensitive short-term bioassays to determine the effects of fossil fuel combustion products on cellular pathways involved in hematopoiesis and immunological processes. Further studies are presently under way to evaluate the effects of in vivo exposure on the progenitor cell function.

In Vivo Studies

Exposure Conditions

We have also performed in vivo inhalation studies with mice acutely exposed to fly ash and silica aerosols and chronically with rat exposure to fly ash alone. In this regard, we have developed technologies for the generation of well-dispersed fly ash aerosols. Our general approach to aerosol generation involves the utilization of a Wright dustfeed mechanism for fly ash deagglomeration and aerosolization followed by a cyclone for separation of larger particles and a krypton-85 discharger for reduction of particulate charge to Boltzman equilibrium (36). In an attempt to improve aerosolization procedures, we compared the efficacy of a fluidized-bed generator to that of the Wright dustfeed system (30). The results of the study indicated that aerosols resulting from the use of the fluidized-bed generator are relatively unstable with time and that deagglomeration is markedly less efficient than that associated with the Wright dustfeed mechanism. Aerosols produced by the Wright dustfeeder had smaller aerodynamic size and broader sized distributions than those resulting from the use of the fluidized bed with or without use of the Wright dustfeed as a feed mechanism. It is for these reasons that we have continued to utilize the Wright dustfeed rather than a fluidized bed for generation of stable, deagglomerated aerosols of fly ash as well as other non-hygroscopic particles.

Acute inhalation studies have been performed with small exposure chambers for periods up to two hours. Such systems employ nose-only technology. Chronic inhalation studies have been performed in immersion chambers for periods up to 180 days for 20 hours per day (36). Size distributions are continuously monitored using a light-scattering particle counter. Size distribution data and aerodynamic data are also obtained using scanning electron microscopic analysis of point-to-plane ESP samples or cascade impactor samples. Total mass measurements are made periodically utilizing filter samples. For acute inhalation studies, we have utilized the finest fly ash fractions of the size-classified, stack-collected fly ash. However, because of the need for relatively large masses of material for chronic inhalation, we have employed size-classified material collected from the hopper of the power plant's electrostatic precipitator.

Macrophage Studies

In acute inhalation studies with mice, using stack-collected fly ash, animals were sacrificed two, six, and fifteen days after exposure, and macrophage function, pulmonary pathology, and progenitor cell kinetics were evaluated (19). Macrophage functional assays indicated a depression in phagocytic capacity of fly-ash-exposed mice at six days and fifteen days after exposure compared to controls. A similar depressed phagocytic activity was observed in silica-exposed animals. Progenitor-cell assays indicated an initial depression in pulmonary alveolar macrophage colonies at two days after exposure and a marked elevation at 15 days after exposure. In contrast to the alterations observed in pulmonary macrophage progenitors, macrophage precursors in bone marrow and spleen were not significantly affected. These data suggest that the elevation in progenitor cell activity appears to be due to recruitment of progenitors from the lung itself, that is, local production. Furthermore, when compared to *in vitro* studies, the replicative capacity of macrophages is readily observed. For example, shortly after acute inhalation exposure, as observed with *in vitro* studies, a depression in progenitor cells was observed. However, two weeks after exposure a marked enhancement was observed, in contrast to *in vitro* studies. Similarly, preliminary studies with i.p. exposure to zinc have indicated that lymphohematopoietic progenitors are less sensitive to *in vivo* exposure than cells exposed *in vitro*.

Further evidence to support the recruitment hypothesis is provided by observations of changes in the relative distributions of particles within phagocytes (19). For the two-, six-, and fifteen-day observation periods, the relative distribution of particles was observed to continually decrease within the cells, suggesting again an enhanced production of phagocytic cells within the lung environment. On the other hand, we have not observed similar effects with long-term, low-level chronic exposure to fly ash aerosols derived from the power plant's electrostatic precipitator. It is not clear whether the difference in biological response reflects either the concentration,--200 milligrams per cubic meter versus 2 milligrams per cubic meter; the difference in species--rat versus mouse; or perhaps, most importantly, the difference of fly ash sources,--stack-collected versus electrostatic-precipitator-hopper-collected ash.

We have also developed techniques for quantification of fly ash deposited in rat lung during the chronic inhalation studies (18). Because of the difficulty in separating particulate matter from lung tissue, we chose to evaluate the utility of elemental analysis as a measure of lung deposition of inhaled fly ash. Selection of the appropriate element required that the following criteria be fulfilled:

1. the elemental analysis should be specific and detection limits should provide a sensitive indicator of lung burden;
2. the tissue content of the element should be low and relatively constant;
3. the dissolution of the element should parallel the particulate mass dissolution; and
4. the element should be uniformly distributed throughout the size range of the fly ash under study.

Under these criteria, only aluminum, silicon, and titanium were considered for further evaluation. Because of the elemental detection limits of atomic absorption spectrophotometry, only aluminum and silicon were chosen for further evaluation. It was found that the analysis of aluminum was more sensitive and less troublesome than that of silicon. Application of the technique to inhalation studies indicated that the lung content of fly ash calculated from the aluminum analysis was in quantitative agreement with calculations based upon available deposition and clearance data.

MUTAGENESIS STUDIES

We have continued to evaluate the mutagenic properties of coal fly ash extracts using the standard Salmonella assay system (6,11). Our studies indicate that the finest fly ash fractions are indeed the most mutagenic in keeping with a model of surface deposition (9). However, the 3.2 μm fraction of fly ash was found to be more mutagenic than the 2.2 μm fraction. At first this was assumed to be due to antimutagens within the fly ash and studies were performed to evaluate the possible role of selenium as the selenite or fluorine as the fluoride. These elements were chosen because of the relatively high concentrations of selenium and fluorine in the finest fly ash fraction compared to that of the 3.2 μm fraction. Also selenium, as the selenite has been demonstrated to be an antimutagen for acetylaminofluorene and its derivatives. Fluoride is generally recognized as an enzyme inhibitor. Addition of these elements to extracts of the 3.2 μm fraction, however, did not alter the mutagenic activity. Thus, we do not have experimental support for the hypothesis that antimutagens are present in the finest fly ash fraction.

It has been suggested by Natusch (31) that the difference in mutagenic activity may be due to differences in chemical absorption of mutagens on the two fly ash fractions. Further evidence for the chemisorption of mutagens on fly ash surfaces is provided by the demonstration of photostability of these compounds. We have irradiated fly ash samples with ultra-violet light, with sunlight, and X-irradiation, and have not observed loss in mutagenic activity. However, heating of the fly ash at temperatures of 200 to 250°C results in loss of approximately one-half of the mutagenic activity, while heating above 300°C results in complete loss of detectable mutagenic activity (11). The biphasic nature of the loss in mutagenic activity with heating indicates the presence of at least two mutagens or classes of mutagens. Most recently, we have demonstrated that the mutagenic loss is due to decomposition of surface-associated materials as opposed to volatilization (22). This important finding further supports the indication of chemisorption of mutagens on fly ash surfaces.

Fly ash collected by the power plant's ESP does not appear to be mutagenic in the Ames test (11). Furthermore, even upon size classification to a size distribution equivalent to our finest, stack-collected fraction, we do not observe mutagenic activity of the electrostatic-precipitator-collected materials. Our studies indicate that temperatures of approximately 100°C may be critical for absorption of mutagens onto fly ash surfaces. Further support for these observations is provided by the calculations of Natusch and Tomkins (32) which indicate that, indeed, the deposition of vapor phase polynuclear aromatic hydrocarbons on fly ash particles is extremely sensitive to temperature changes around 100°C.

Studies of the comparative efficiency of mutagen extraction by a variety of solvents have been performed. We have found that normal saline is a very poor extractant of mutagenic activity, whereas horse serum and serum from other species are fairly efficient extractants of fly ash mutagens (6). Our data indicate the formation of a mutagen-serum protein complex that can be isolated from the serum, although it is available to the bacterial genome. Further studies indicated that albumen alone is nearly as efficient as the total serum in extracting mutagens from the fly ash (5). Direct extraction of fly ash with dimethylsulfoxide and sonication results in the greatest detectable levels of mutagens.

We have not identified the chemical composition of the mutagens of fly ash, although most recent studies using acidic, neutral, and basic aqueous extracts further support the indication that the mutagens in fly ash are not inorganic. Acidic aqueous fractions were not mutagenic, whereas basic aqueous fractions contained approximately one-half of the mutagens extractable with dimethylsulfoxide. These results indicate that a significant portion of the mutagenic activity in coal fly ash can be accounted for by the presence of weak organic acids.

To evaluate the carcinogenic potential of coal fly ash, we have developed a modification of the tracheal implant system described by Griesemer et al. (21). For these studies, we have packaged fly ash in 0.2 μm Nuclepore filters. This approach allows for the slow release of mutagens to the sensitive tracheal epithelial cells and minimizes risks for local tissue damage and toxicity. We have employed the bacterial mutagenesis system to monitor the release of mutagens from the fly ash in the tracheal implants (3). Studies are now underway to evaluate the carcinogenic potential of the fly ash using the tracheal implant system.

CONCLUSIONS

In conclusion, our results demonstrate the extreme complexity of coal fly ash in terms of matrix composition, morphological appearance, surface trace element and organic chemical composition. Assays are now evolving for the measurement of the potential immunotoxicity of fly ash. Acute inhalation studies have demonstrated that coal fly ash may be equally as toxic as alpha-quartz to the pulmonary alveolar macrophage. The feasibility of application of sophisticated cloning techniques for the evaluation of potential lympohematopoetic effects from complex mixtures has been demonstrated. Mutagens in coal fly ash appear to be absorbed to fly ash surfaces and hence may exist in the environment for relatively long periods of time. Techniques are now being developed to evaluate the carcinogenic potential of coal fly ash utilizing a combination of bacterial mutagenesis assays and tracheal implant carcinogenesis assays. Detailed chemical, morphological, and toxicological analyses indicate the utility of coal fly ash as a model complex mixture.

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FOOTNOTE

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ELEMENTAL ANALYSIS FOR ENVIRONMENTAL ASSESSMENT MEASUREMENTS

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ABSTRACT

Several recent studies have been initiated to evaluate specific areas of the Inorganic Analytical Protocol for Environmental Assessment Measurements. The results of a portion of this work which focuses on the elemental analysis scheme are described here. Emphasis is placed on the Level 1 scheme and the effects of organic matter on elemental determinations. In conjunction with this work, several sample preparation techniques were evaluated. The preparation methods are discussed in terms of their potential utility for environmental assessment measurements.

INTRODUCTION

Elemental analysis plays a key role in all phases of an Environmental Assessment (EA) Measurement Program. In the IERL-RTP three-tiered approach (2) to the EA of stationary sources, the first phase (Level 1) is devoted to comprehensiveness. The Level 1 study cannot rely on currently known toxic species or on prior knowledge of the source for selection of a set of parameters to be determined. The constraint which disallows these assumptions at the outset of the measurement program is meant to preclude the possibility of missing potential hazards. The objective of the Level 1 elemental analysis scheme is to provide a complete elemental survey of the source emissions in order to define the hazard potential of inorganic emissions and to identify possible control needs.

Subsequent phases of the assessment seek more specific determinations. The elemental analysis requirements of the second phase, Level 2, are very specific and are usually limited to a few elemental determinations. Level 2 elemental data are needed to confirm and refine Level 1 results that flag emission problems. Elemental analyses in conjunction with separation schemes can also be used for indirect compound speciation at Level 2. Elemental analysis may also be required in the final phase, Level 3, which entails process and control monitoring to define the temporal variation of source emissions.

A series of studies have recently been conducted to evaluate several areas of the Level 1 elemental analysis scheme. As part of this research, the effects of organic matter on elemental determinations by spark source mass spectrography (SSMS), the primary Level 1 elemental analysis technique(4), were investigated. In addition, several sample preparation techniques were evaluated in terms of efficacious elimination of organic matter without resultant elemental losses or contamination. The utilities of the methods as preparation techniques for atomic absorption spectrometry (AAS) measurements (principally Hg) were also assessed.

A summary of the results of these investigations is presented herein. While emphasis is placed on Level 1 methods, the preparation studies have definite implications regarding Level 2 analyses.

EXPERIMENTAL

Test Sample Fabrication

The test samples used to evaluate the effects of organic matter on Level 1 elemental analyses were prepared from spiked NBS SRM 1633 Fly Ash and an organic mixture. The fabrication procedure consisted of three steps: First, the standard fly ash was spiked by the addition of solid compounds containing nine elements (U, Pb, Ce, I, Ag, Se, As, Sc, and Cl). Because previous experience had shown that inhomogeneity is likely to result when a sample is diluted by more than 10:1, a multiple dilution technique was utilized in preparing the fly ash starting material. In the next fabrication step, an organic mixture was prepared consisting of 80% ascorbic acid, 10% benzoic acid and 10% carbon. Each of these materials was screened for elemental impurities and found to be sufficiently pure for the present purpose. The test samples were then fabricated from the fly ash and organic mixtures. Several sets of four dilutions were made. They are referred to throughout this paper as containing 0%, 10%, 50% and 90% organic matter. However, it should be noted that the NBS fly ash proper does contain some residual organic matter. Nevertheless, this sample is considered to be the baseline for 0% or negligible organic content. The theoretical concentration values for the spiked NBS fly ash are given in Table 1.

Sample Preparation Methods

The four sample preparation methods investigated in this study are the Parr oxygen bomb technique (PB), the low temperature plasma ashing method (LTA), a hydrofluoric acid digestion bomb procedure (HFB), and a mineral acid extraction (AE). All four methods were applied to the fabricated test samples and the PB and LTA methods were also applied to NBS SRM 1632 coal.

The PB procedures were performed in accordance with the Level 1 manual (4). The oxygen bomb apparatus was purchased from Parr Instrument Co. and modified in house. The modifications consisted of replacing the standard electrodes with electrodes fabricated from 97% platinum and 3% rhodium and fitting the bomb with a fused silica liner with cover.

The LTA preparations followed the instrument manufacturer's recommendations (5). An LFE Model LTA-302 was used for all in-house preparations. The instrument was operated at an rf power level of 300 watts with an O_2 flow of 200 cc/min. The ashing times varied from 6 to 12 hours. All samples were ashed to constant weight and the percent ash calculated. The percent ash values were used to adjust the analytical results to reflect the original sample weight.

TABLE 1

Theoretical Values for NBS SRM 1633 Fly Ash Including Spiked Elements

Element	Concentration (ppm)	Element	Concentration (ppm)
U*	360	Y	50
Th	20	Sr	1380
Pb*	1160	Rb	102
Tl	3.2	Br	9.8
W	3.7	Se*	257
Ta	1.5	As*	980
Hf	6.4	Zn	176
Lu	0.81	Cu	104
Yb	5.7	Ni	80
Tb	1.5	Co	34
Eu	2.0	Fe	5.0 (%)
Sm	10.0	Mn	404
Ce*	2390	Cr	103
La	67	V	191
Ba	2200	Ti	6030
Cs	7.0	Sc*	326
I*	79	Ca	3.8 (%)
Sb	5.6	K	1.3 (%)
Cd	1.2	Cl*	685
Ag*	1960	Si	17 (%)
Zr	245	Al	10 (%)
		Mg	1.5 (%)
		Na	2610
		Be	9.7

* - Elements which were added (as compounds) to the fly ash.

The HFB procedure studied was proposed by Hartstein et al. (3). This procedure is similar to the classical HF bomb technique (1) routinely employed in the preparation of mineral samples. However, two steps have been added which make the technique much more amenable to the present needs. First, a nitric acid digestion step is employed to aid removal of organic matter prior to HF digestion which is intended to be a dissolution step. After HF digestion the resulting sample is treated with boric acid to further aid dissolution. The procedure utilizes a teflon-lined stainless steel bomb (also available from Parr Instrument Co.).

The AE procedure was performed according to the Level 1 acid extraction method for solid samples and particulate filter samples to be subjected to cold vapor mercury analysis (4). This procedure specifies a 6-hour extraction in a 4 to 1 mixture of HNO_3 to HCl, respectively, followed by filtration and dilution to 100 mL.

Elemental Analysis

SSMS analyses were performed on the four test samples by three different laboratories. The combination of replicate analyses and the number of laboratories involved yields 24 possible data sets for the four samples used in the evaluation of organic matter on SSMS measurements. No effort was made to specify the SSMS procedures to be used. The choices of instrument operating conditions, electrode preparation techniques, standardization procedures, interpretation and quantitation methods, etc., were left completely up to the individual laboratories. The laboratories were selected to represent a cross-section of instrument types and procedural details. SSMS analyses were also performed on NBS SRM 1632 coal samples after preparation by the PB and the LTA techniques. Selected samples prepared by these techniques were analyzed by the different participating laboratories.

All atomic absorption spectrometry (AAS) analyses were performed in-house on a Perkin Elmer Model 460 equipped for both flame and flameless methods. The cold vapor technique described in the Level 1 manual (4) was used for all mercury determinations. Other elemental determinations by AAS employed either standard flame or graphite furnace (Model HGA 2100) techniques as appropriate.

Selected elemental determinations were also made by Inductively Coupled Argon Plasma Spectroscopy (ICAPS). A Jarrell-Ash Model 1160 Plasma AtomComp$^{(TM)}$ equipped with 24 fixed wavelength channels and a Mark V N+1 channel was used for all ICAPS analyses.

RESULTS AND DISCUSSION

Organic Effects on SSMS

Organic constituents of a sample can potentially affect SSMS elemental determinations in several ways. The general areas in which organics can cause problems include:

- Electrode Preparation
- Data Collection
- Qualitative Interpretation
- Quantitation
- Detection

The nature of the effects and the degree to which the analysis may suffer are dependent on the specific organic material present. A consideration of the vast number of different organic matrices that can be envisioned clearly places constraints on the evaluation study at the outset. It would be an extraordinarily costly if not futile effort that endeavors to precisely determine all effects produced by a host of matrices. Therefore, the effects that can be reasonably studied are limited by the choice of test materials.

The test samples employed in this study allowed some specific areas to be addressed. The organic matrix chosen contributed only carbon, hydrogen and oxygen to the test samples. This matrix produces an abundance of molecular spectra that are typical of those which might be produced by organic matter in environmental samples. Thus, the effects of molecular fragments on qualitative interpretation and consequently on quantitation could be observed. Also, the organic matrix present in the test samples allowed reasonable simulation of the sparking (burning) characteristics of incompletely combusted matter and thereby allowed some evaluation of the effects on data collection. The only effect on detection expected from the organic constituent of the test samples used is that of simple dilution which alters the ultimate detectability but does not alter the sensitivity. The remaining area, electrode preparation effects, was not addressed in this study. Electrode preparation problems are immediately obvious to the analyst; if suitable electrodes cannot be prepared, severe matrix problems are indicated.

The 0% sample was used as the baseline for all effects considered. Figure 1 shows the results obtained from all laboratories on the 0% sample. This histogram plots elements versus A (= log (X_m/X_a)) where X_m is the concentration reported and X_a is the actual concentration. For example, all determinations in the range 0.0 to ± 0.10 are within 25% of their true values. The vertical lines indicate deviations from the actual values by factors of 2 (A = ± 0.30) and 3 (A = ± 0.48). The results reported by Laboratory B for the 0%, 10%, and 50% samples, which are representative of the results reported by all three laboratories, are shown in Figure 2. As seen from Figure 2, there is little difference in the results for these three samples. Although the data for the 50% organic sample indicate a slight positive bias, it does not exceed the precision of the measurement. Also, the results from the other laboratories do not substantiate any positive bias with increasing organic content.

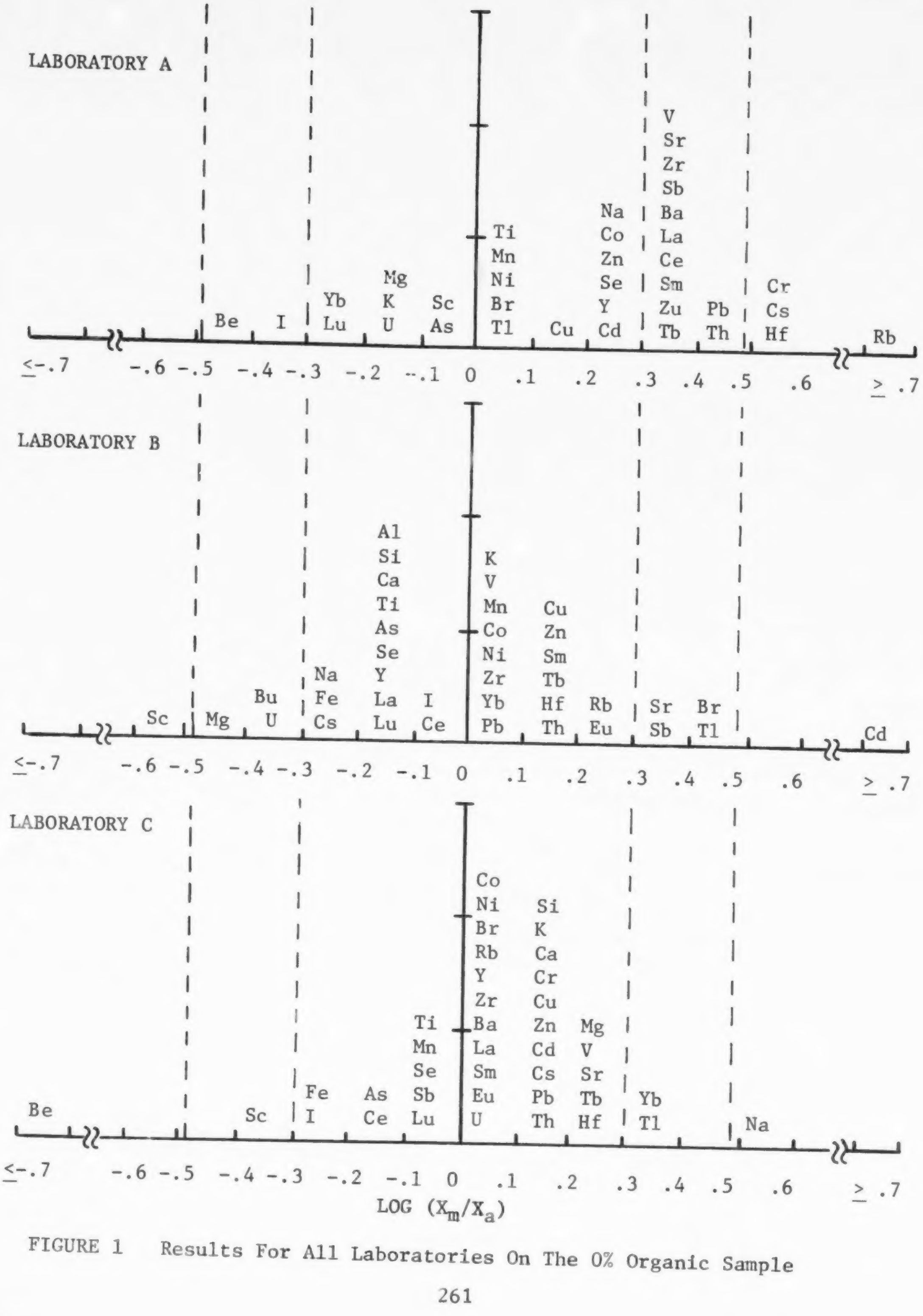

FIGURE 1 Results For All Laboratories On The 0% Organic Sample

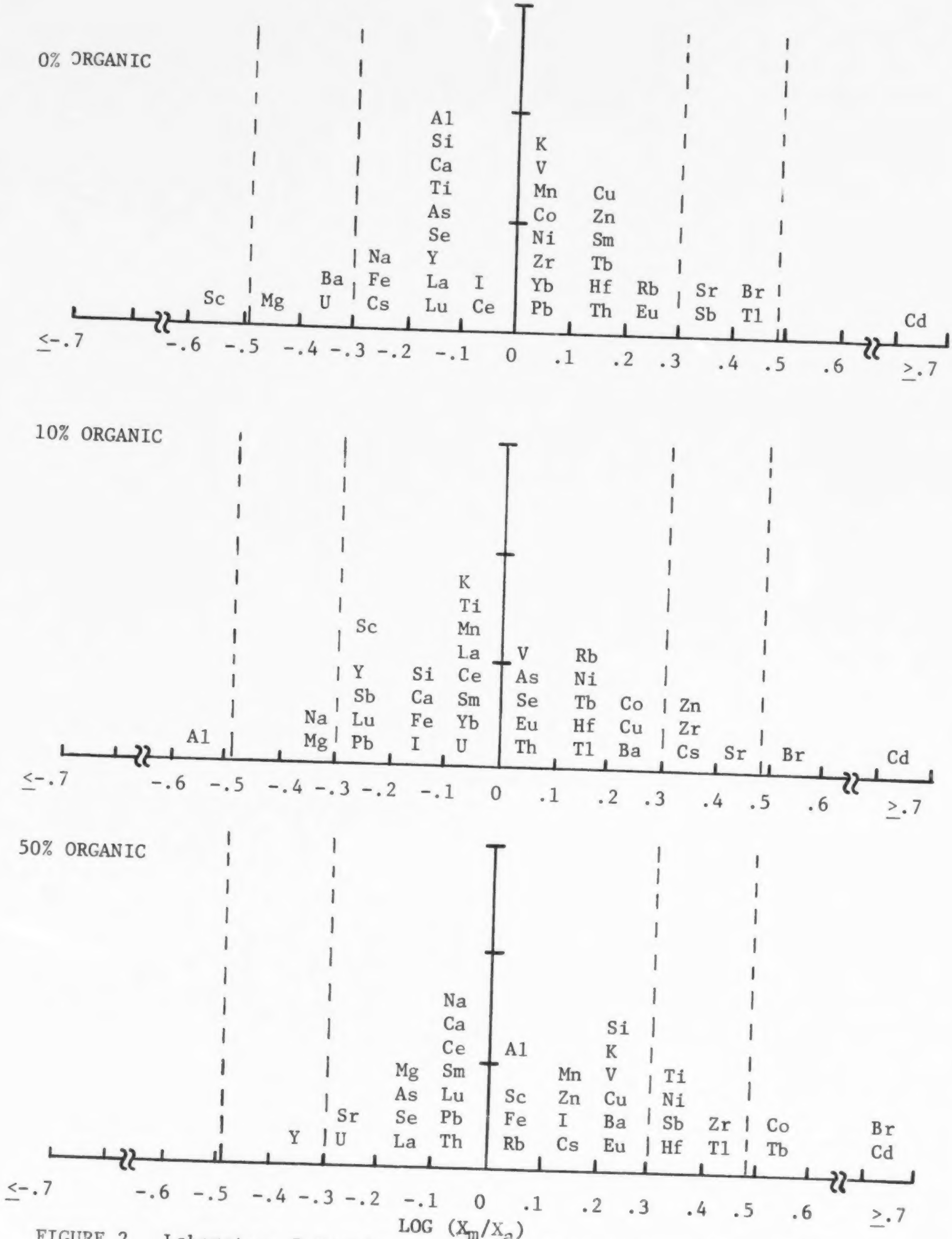

FIGURE 2 Laboratory B Results On The 0%, 10% and 50% Organic Samples

SSMS analysis of the 90% organic sample was problematic for all laboratories. While electrode preparation problems were not reported, data collection and photoplate analysis problems with this sample were encountered. Of the six samples submitted for analysis, only one data set on the unaltered 90% sample was obtained. Although this data set did contain substantially more unacceptable values and showed considerable scatter, valid conclusions regarding organic interferences cannot be drawn from the results of this single sample. Excessive molecular spectra were cited as a cause for concern with this sample.

The photoplates produced by all laboratories revealed increased molecular spectra with increasing organic content. Molecular spectra are typically produced from organic constituents in the sample in fairly consistent patterns or "runs". A hydrocarbon run usually takes the form C_nH_{n+x} ($X=0$, ± 1, ± 2, ± 3, etc.). The spectral lines for $X = 0$ are the most intense and the intensity rapidly decreases with increasing X. Hydrocarbon runs resulting from the organic constituent of our test samples were easily discernible by all laboratories by visual inspection of the photoplate as evidenced by the fact that all reports included comments to this effect. In fact, the observation of these spectra provided the analysts with a guideline for sample preparation requirements. In general, if the molecular spectra were judged excessive by the individual analysts, sample preparation steps were undertaken.

The data resulting from this set of experiments substantiate the validity of the "molecular spectra guidelines" for determining sample preparation requirements; however, this may not be the most cost-effective procedure for deducing the necessity for sample preparation.

It should be noted that while the vast majority of the determinations fell within a factor of 3 of the actual value, some did exceed this range. The factor of 3 is used as a breakpoint for accuracy evaluations in view of Level 1 objectives. Because a factor of 3 allows no margin for error in other phases of the measurement endeavor, deviations approaching a factor of 3 should be considered marginally acceptable. The data obtained during this study revealed determinations outside the acceptable range for all participating laboratories. Although the unacceptable determinations were consistent (some elements) for the different samples within the same laboratory, an interlaboratory comparison reveals few consistent problems. It was observed that beryllium appears to be a consistent problem; however, the sign (±A) of the error was not the same for all laboratories. These results indicate that specific procedural differences are probably responsible for the observed problems; there is no evidence suggesting that organics were responsible for the outlying determinations.

Sample Preparation Methods

In this phase of the study, several preparation methods were evaluated in terms of 1) their abilities to eliminate organic constituents without concomitant elemental losses, and 2) the comprehensiveness of their dissolution capabilities. Although the SSMS analysis does not require sample dissolution, liquid samples are amenable to SSMS analysis. Conversely, other elemental analysis techniques, especially the likely choices for Level 2 analysis, do require solution samples. If multielement analyses are necessary at Level 2, a comprehensive dissolution technique for solid samples would be desirable.

The preparation methods studied were:

- Parr Oxygen Bomb Combustion (PB)
- Low Temperature Plasma Ashing (LTA)
- Acid Digestion Bombing (HFB)
- HNO_3/HCl Extraction (AE)

The PB procedure was performed according to the Level 1 procedures manual (4). It should be noted that two slightly different PB procedures are specified. The necessity for two procedures arises because of the possible "mineral" residues after combustion. While these residues must be digested and removed prior to the cold vapor mercury analysis, they should be retained and combined with the resulting solution prior to SSMS analysis. In order to avoid subsequent aliquotting problems, we recommend that the entire sample (all solution and all residue) be used for the SSMS analysis. This eliminates inaccuracies or biases produced by aliquotting a solid/solution mixture and reduces contamination introduced by volume adjustment and subsequent aliquotting.

The SSMS results for the Parr bombed 90% organic sample are shown in Table 2. The set of elements shown in Table 2 were used for all preparation technique evaluations when SSMS was the analytical finish. With some exceptions, these elements represent all of the well characterized concentration data for NBS SRM 1633 and 1634. The few exceptions were elements determined to be problematic during the baseline study described above and, therefore, are inappropriate for use here.

As seen from Table 2 the data resulting from the PB preparation satisfy the Level 1 requirements. It is clear from this data set that elemental losses are not a problem with the PB method. Inspection of the photoplates resulting from this analysis indicates that the organic matrix was effectively eliminated.

We note that, ostensibly because of the mineral nature of the inorganic constituents, residues did result on combustion of this sample. The data presented in Table 2 correspond to sample electrodes prepared from the combination of the solid and liquid fractions. The SSMS and AAS results obtained on the solution only portion of this sample are shown in Table 3 and Table 4, respectively. The SSMS data shown for the two laboratories consistently illustrate the elemental losses which occur upon discarding the solid residue. The AAS data, given in Table 4, confirm the SSMS results. The mean concentration values in Table 4 were derived by first normalizing the results for all (0%-90%) test

TABLE 2

SSMS Results on Oxygen Bombed 90% Organic Sample

Element	Expected Value (ppm)	Measured Value (ppm)	Effect*
U	36.0	34	✓
Th	2.0	3.3	✓
Pb	116	120	✓
Yb	0.57	ND	o
Eu	0.20	0.12	✓
Sm	1.0	0.83	✓
Ce	239	260	✓
La	5.7	13	✓
Ba	220	430	✓
Cs	0.70	0.72	✓
I	7.9	5.0	✓
Sb	0.56	0.84	✓
Cd	0.12	0.14	✓
Zr	24.5	35	✓
Br	0.98	0.52	✓
Se	25.7	38	✓
As	98	140	✓
Zn	17.6	20	✓
Cu	10.4	16	✓
Ni	8.0	16	✓
Co	3.4	6.0	✓
Fe (%)	0.58	0.88	✓
Mn	40.4	53	✓
Cr	10.3	13	✓
V	19.1	24	✓
Ti	603	1000	✓
Ca	3800	4900	✓
K	1300	670	✓
Si (%)	1.7	2.1	✓
Al (%)	1.0	MC	o
Mg	1500	600	✓
Na	261	240	✓

*✓ indicates within a factor of 2; ✓± indicates high (+) or low (-) but within a factor of 3; + or - denote results unacceptably high (+) or low (-), respectively; o means no evaluation made.

TABLE 3

SSMS Results On Oxygen Bomb Solution Of 90% Organic Samples

Element	Expected Value (ppm)	LAB X Measured Value (ppm)	LAB X Effect*	LAB Y Measured Value (ppm)	LAB Y Effect*
U	36.0	6.9	–	4.4	–
Th	2.0	0.4	–	ND	o
Pb	116	21	–	23	–
Yb	0.57	ND	o	ND	o
Eu	0.20	ND	o	ND	o
Sm	1.0	0.30	–	ND	o
Ce	239	38	–	17	–
La	6.7	0.63	–	0.72	–
Ba	220	44	–	19	–
Cs	0.70	ND	o	0.082	–
I	7.9	0.30	–	1.7	–
Sb	0.56	0.30	√	0.18	–
Cd	0.12	0.06	√	0.037	–
Zr	24.5	1.9	–	1.2	–
Br	0.98	0.50	√	0.54	√
Se	25.7	4.1	–	8.6	–
As	98.0	69	√	44	√–
Zn	17.6	8.8	√	7.1	√–
Cu	10.4	4.3	√–	1.0	–
Ni	8.0	1.4	–	4.7	√
Co	3.4	0.60	–	0.26	–
Fe	5800	750	–	220	–
Mn	40.4	14	–	2.4	–
Cr	10.3	1.9	–	1.9	–
V	19.1	4.7	–	1.9	–
Ti	603	44	–	18	–
Ca	3800	1400	√–	250	–
K	1300	1800	√	120	–
Si (%)	1.7	0.21	–	0.021	–
Al (%)	1.0	0.31	–	0.27	–
Mg	1500	3400	√+	110	–
Na	261	280	√	320	√

*√ indicates within a factor of 2; √± indicates high (+) or low (-) but within a factor of 3; + or - denote results unacceptably high (+) or low (-), respectively; o means no evaluation made.

TABLE 4

AAS Results (Normalized) on the Oxygen Bomb Solutions
Of All (0-90%) Organic Samples*

Element	Expected Value (ppm)	Mean Value (ppm)	% RSD	% D
Pb	1160	444	19	-62
Hg	0.11	0.12	- **	9
Cd	1.2	2.1	2.0	75
Se	257	106	51	-59
As	980	1270	26	29
Zn	176	45	3.2	-74
Cu	104	31	22	-70
Ni	80	26	30	-68
Mn	404	114	45	-72
Cr	103	13	30	-87
V	191	102	10	-47

* Data were normalized to the 0% sample concentrations prior to statistical analysis.

** Hg concentrations in the organic diluted samples were not detectable.

samples to the 0% organic level and then subjecting the data to statistical analysis. The normalization to 0% organic content creates the factor of ten difference in expected values (and mean values for AAS) seen in these two tables. The differences between the AAS and SSMS values observed are well within the combined experimental errors of the two techniques.

The AAS data also indicate the utility of the PB method for mercury determinations even when the inorganic matrix is, in general, refractory. It is equally interesting that arsenic was well determined on the PB solution by both techniques. As noted in Table 4, no statistical analysis could be performed on the mercury data. Since the mercury concentration of the 0% sample is very near the experienced detection limit, any organic dilution resulted in non-detectable mercury values. This result is simply a consequence of the restricted sample size employed. The sample size limitation is the only drawback of the PB technique experienced during this study. While contamination effects were not specifically addressed, none were noted.

The results obtained from two laboratories on the 90% sample prepared by LTA are shown in Table 5. By and large, these data satisfy Level 1 requirements, but some inconsistencies between the two laboratories are noted. The values reported for zinc and zirconium by one laboratory are abnormally high and suggest ambient environmental contamination. No rationalization can be given for the low values reported for cerium and titanium. Inspection of the photoplates produced by each laboratory reveals very clean essentially organic free spectra. The results from both laboratories indicate losses due to preparation for iodine and bromine.

Table 6 compares the SSMS results on NBS SRM 1632 coal prepared by the PB and the LTA methods. As seen from these data, the two methods yield comparable results. Halogen losses are observed for both techniques with this sample. This differs from the previous results which indicated that no losses were incurred via the PB preparation. This discrepancy suggests that matrix effects may be a problem with halogen determinations by SSMS. The inconsistency observed for the silicon determination is not unexpected since major component measurements are difficult to make by standard SSMS techniques. Inspection of the photoplates resulting from each preparation of this sample indicated adequate organic removal for both techniques. The LTA technique again produced the cleaner photoplate.

The SSMS data for the 50% sample prepared by HFB are presented in Table 7. The 50% sample was selected for study by this procedure because it represents the maximum tolerable organic content (6) according to the manufacturer of the bomb. The manufacturer's warning arises from the explosion potential produced by samples containing primarily (>50%) organic constituents. It should be noted that this recommendation applies to the standard HF procedure for which the bomb was designed. The authors of the modified procedure (3), which uses a nitric acid digestion step to remove organics, did not address this issue specifically but the samples prepared (coal samples) did contain high organic content.

TABLE 5

SSMS Results on LTA of 90% Organic Samples

Element	Expected Value (ppm)	LAB 1		LAB 2	
		Measured Value (ppm)	Effect*	Measured Value (ppm)	Effect*
U	36.0	12.0	✓-	42.0	✓
Th	2.0	2.0	✓	3.0	✓
Pb	116.0	124.0	✓	250.0	✓+
Yb	0.57	1.2	✓	1.8	✓+
Eu	0.20	0.30	✓	0.21	✓
Sm	1.0	1.4	✓	1.2	✓
Ce	239.0	16.0	-	220.0	✓
La	6.7	3.5	✓	6.1	✓
Ba	220.0	380.0	✓	280.0	✓
Cs	0.70	1.1	✓	0.98	✓
I	7.9	> 5.0	o	0.48	-
Sb	0.56	1.4	✓	0.38	✓
Cd	0.12	4.8	+	0.16	✓
Zr	24.5	77.0	+	20	✓
Br	0.98	> 1.4	o	0.29	-
Se	25.7	21.0	✓	10.0	✓-
As	98.0	32.0	✓-	33.0	✓-
Zn	17.6	75.0	+	8.8	✓
Cu	10.4	23.0	✓	6.8	✓
Ni	8.0	18.0	✓+	5.3	✓
Co	3.4	11.0	✓+	2.4	✓
Fe (%)	0.5	0.35	✓	0.20	✓-
Mn	40.4	37.0	✓	32.0	✓
Cr	10.3	> 31.0	o	6.7	✓
V	19.1	28.0	✓	18.0	✓
Ti	603.0	160.0	-	580.0	✓
Ca	3800.0	2000.0	✓	3800.0	✓
K	1300.0	1900.0	✓	540.0	✓-
Si (%)	1.7	1.9	✓	0.96	✓
Al (%)	1.0	1.2	✓	MC	o
Mg	1500.0	~ 1500.0	✓	600.0	✓-
Na	261.0	240.0	✓	640.0	✓+

*✓ indicates within a factor of 2; ✓± indicates high (+) or low (-) but within a factor of 3; + or - denote results unacceptably high (+) or low (-), respectively; o means no evaluation made.

TABLE 6

SSMS Results On Prepared NBS SRM 1632 Coal

Element	Accepted Value (ppm)	LTA		Oxygen Bomb	
		Measured Value (ppm)	Effect*	Measured Value (ppm)	Effect*
U	1.4	1.8	√	0.56	√-
Th	3.0	5.4	√	2.7	√
Pb	30.0	21.0	√	56.0	√
Yb	0.7	ND	o	ND	o
Eu	0.33	ND	o	ND	o
Sm	1.7	ND	o	3.1	√
Ce	19.5	12.0	√	16.0	√
La	10.7	9.6	√	13.0	√
Ba	352.0	270.0	√	430.0	√
Cs	1.4	1.1	√	0.74	√
Sb	3.9	3.0	√	3.4	√
Cd	0.19	0.11	√	0.073	√-
Br	19.3	3.1	-	2.0	-
Se	2.9	3.5	√	2.3	√
As	5.9	13.0	√	8.6	√
Zn	37.0	53.0	√	43.0	√
Cu	18.0	45.0	√+	32.0	√
Ni	15.0	25.0	√	33.0	√
Co	5.7	9.1	√	6.1	√
Fe (%)	0.87	1.3	√	0.90	√
Mn	40.0	40.0	√	53.0	√
Cr	20.2	20.0	√	26.0	√
V	35.0	73.0	√	24.0	√
Ti	1100.0	1500.0	√	1000.0	√
Ca (%)	0.43	0.71	√	0.5	√
K (%)	0.28	0.21	√	0.3	√
Si (%)	3.2	12.0	+	4.4	√
Al (%)	1.85	MC	o	MC	o
Mg (%)	0.2	0.20	√	0.063	-
Na	414.0	1500.0	+	480.0	√

*√ indicates within a factor of 2; √± indicates high (+) or low (-) but within a factor of 3; + or - denote results unacceptably high (+) or low (-), respectively; o means no evaluation made.

TABLE 7

SSMS Results On HF Bombed 50% Organic Sample

Element	Expected Value (ppm)	Measured Value (ppm)	Effect*
U	180	210	✓
Th	10	33	+
Pb	580	1200	✓+
Yb	2.9	7.0	✓+
Eu	1.0	2.5	✓+
Sm	5.0	15	✓+
Ce	1200	2500	✓+
La	33.5	65	✓
Ba	1100	3100	✓+
Cs	3.5	7.5	✓+
I	39.5	7.5	–
Sb	2.8	2.9	✓
Cd	0.60	0.38	✓
Zr	123	290	✓+
Br	4.9	1.2	–
Se	129	41	–
As	490	380	✓
Zn	88	49	✓
Cu	52	65	✓
Ni	40	55	✓
Co	17	19	✓
Fe (%)	2.9	1.6	✓
Mn	202	320	✓
Cr	51.5	70	✓
V	95.5	95	✓
Ti	3020	2200	✓
Ca (%)	1.9	1.8	✓
K (%)	0.65	0.31	✓–
Si (%)	8.5	0.0039	✓–
Al (%)	5.0	MC	o
Mg (%)	0.75	0.29	✓–
Na	1300	750	✓

*✓ indicates within a factor of 2; ✓± indicates high (+) or low (-) but within a factor of 3; + or - denote results unacceptably high (+) or low (-), respectively; o means no evaluation made.

Advisedly, the present study did not attempt to pursue this limitation of the technique.

In general, as seen from Table 7, the SSMS results for this preparation are in reasonable agreement with the expected values. The observed values for thorium, iodine, bromine, selenium and silicon are notable discrepancies. The loss of silicon via the formation of volatile silicon hexafluoride was expected; however, similar interactions apparently occur between the HF and iodine, bromine, and selenium which consequently result in losses of these elements as well. While the value observed for thorium is only slightly outside the acceptable range (well outside the desired range), it emphasizes the general trend of positive bias observed in the high atomic weight region for this sample. No further investigation of this trend was attempted in the present study.

The modified HFB procedure was also applied to Fluidized Bed Combustion ash samples for AAS analyses in order to determine a materials mass balance. Because the samples involved did not contain significant organic constituents, the nitric acid step was omitted. The boric acid step was included since sample dissolution is mandatory for AAS analysis. While this study confirmed the dissolution capabilities of the modified HFB procedure, some problems were noted. Specifically, the use of boric acid to aid dissolution introduces a severe interference to the graphite furnace AAS determination of trace element constituents. While the boric acid has little effect on the standard flame AAS determination, the graphite furnace AAS measurements required for ultra-trace constituents are precluded by the effects of the boric acid step.

An acid extraction (AE) procedure that uses 4 parts nitric acid to 1 part hydrochloric acid as the extraction medium was also evaluated. In the Level 1 scheme (4), this extraction procedure is employed to prepare all primarily inorganic (non-combustible) samples for cold vapor mercury analysis. This method is also used to prepare particulate filter samples for SSMS analysis in situations where the particulate cannot be removed from the filter substrate.

The elemental analysis results obtained on NBS SRM 1633 fly ash prepared by the AE procedure are shown in Table 8. While most of the data shown in Table 8 are ICAPS determinations, some of the data are AAS measurements. The AAS data were used to supplement the ICAPS results for elements that were either not detected by ICAPS or the presence of interferences to the technique were obvious. As seen from Table 8, the AE procedure did extract substantial quantities of most elements. With some exceptions, quantities sufficient to meet Level 1 requirements were extracted; however, the data clearly show a negative bias owing to incomplete dissolution. This bias from preparation, combined with the limitations of the Level 1 SSMS procedure, could lead to unacceptable SSMS results. It should be noted that the AE in conjunction with cold vapor AAS yields results for mercury that are adequate for Level 1 and Level 2. However, with a few exceptions, the AE method appears inadequate preparation for Level 2 analyses.

TABLE 8

Elemental Results on NBS SRM 1633 Fly Ash Prepared By The AE Procedure*

	Accepted Value (ppm)	Measured Value (ppm)	Level 1 Requirements	Level 2 Requirements§
Al (%)	12.7	5.4	✓–	–
Ba	2700	2710	✓	✓
Ca	4.7	2.6†	✓	–
Cd	1.45	2.0†	✓	o
Co	41.5	27	✓	–
Cr	131	77	✓	–
Cu	128	131	✓	✓
Fe (%)	6.2	3.7	✓	–
Hg	0.14	0.11†	✓	✓
K (%)	1.72	0.25†	–	–
Mg (%)	1.8	0.92†	✓	–
Mn	493	283†	✓	–
Ni	98	46	✓	–
Pb	70	22	–	–
Si (%)	21	0.0253	–	–
Ti	7400	2760	✓	–
V	214	214	✓	✓
Zn	210	154	✓	–

*Determined by ICAPS unless noted otherwise.

†Determined by AAS.

§An accuracy of ± 25% is assumed for Level 2 requirements.

CONCLUSIONS

SSMS analyses were conducted by three laboratories on a set of test samples containing various amounts of organic matter in an effort to evaluate the effects of organics on the SSMS results. These organic test samples and selected NBS SRM materials were used to evaluate several sample preparation methods. The following conclusions are drawn from the organic effects study:

- Organic components of a sample can adversely affect, and in some cases preclude, SSMS data collection.
- Organic molecular spectra can hinder qualitative interpretation of the elemental spectra.
- Molecular spectra produced by organic constituents are readily discernible by SSMS analysts.
- Interference from the coincidence of organic and elemental spectra was not a problem.
- For the test organic matrix used, 50% organic content was tolerable.

It is clear from the foregoing discussion that, in some cases, preparation prior to SSMS analysis is necessary. In addition, the delineation of sample preparation methods suitable for Level 2 elemental analyses is highly desired. The following conclusions are drawn from the sample preparation study:

- The Parr Oxygen Bomb technique eliminates organic constituents adequately for SSMS analysis. Although this procedure can result in a mixed phase sample, it appears to be the most comprehensive SSMS preparation technique when all residues can be collected. While the method is inadequate for the dissolution of many elements, it is an excellent preparation method for cold vapor mercury analysis. In terms of the Level 1 elemental analysis scheme, the only significant drawback to this method is the limited sample size that can be accommodated by the standard apparatus.
- The Low Temperature Plasma Ashing technique is superior to any technique studied for the elimination of organic matter. However, when nominally "volatile" elements are of interest, the method is questionable; halogen losses represent a fundamental problem with the method. A major advantage of the method is the ability to accommodate large sample sizes. Disadvantages to the method include: its vulnerability to ambient environmental contamination and the long ashing times required for some samples.

- The Modified Hydrofluoric Acid Digestion Bomb technique is the most comprehensive dissolution technique studied. With the addition of a fuming nitric acid step, organic removal can also be accomplished. Concentration steps achieved by solution evaporation will result in the loss of silicon; halogen losses also occur. While the boric acid step aids sample dissolution, the resulting matrix interferes with graphite furnace AAS measurements.

- The Level 1 Acid Extraction technique is adequate for the preparation of particulate samples for cold vapor mercury analysis. Some other elements can also be successfully extracted by this method; however, the method has limited application potential for Level 2 analysis.

In summary, the Level 1 elemental analysis scheme was found to be reliable and to provide the kind of information desired from the Level 1 study. Problems were noted with some SSMS determinations and additional work in this area is recommended. The preparation of particulate filter samples for SSMS analysis by the AE procedure may bias the resulting data.

The HFB method shows great promise as a Level 2 preparation procedure for analytical techniques requiring solution samples. Additional work in this area is needed.

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LEVEL 2 INORGANIC SAMPLING AND ANALYSIS METHODOLOGY APPLIED TO FGD SYSTEMS

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TRW DSSG

ABSTRACT

During the past 3 years TRW has been involved in the development of Level 2 inorganic sampling and analysis methodologies. A comprehensive analysis scheme was developed to meet both the trace element analysis and the compound identification needs of IERL/PMB.

Under several EPA programs TRW has applied this analysis scheme, which uses such techniques as TGA/DSC, optical microscopy, SEM-EDX, AAS, ICP, FTIR, XRD, ESCA, and SIMS, to samples from SASS and Method 5 type trains and impactor systems. This paper will present a discussion of the application of several of these inorganic sampling and analysis methods to particulate samples from oil-and coal-fired boilers equipped with FGD systems. Data will be presented on the trace element enrichment across the scrubber, surface characterization of size classified particulate matter, and compound identification using FTIR.

INTRODUCTION

The development of more stringent air quality standards is changing the inorganic analysis requirements for environmental samples. There is a growing trend in government regulations and academic interest to go beyond trace elemental analysis to compound identification. Unfortunately, no single inorganic method can identify all compounds in all samples. Added to this problem is the fact that surface and bulk composition might be widely different. In order to meet these requirements and characterize the inorganic portion of any environmental sample, specific data needs must be defined and then appropriate analytical tools integrated into an analysis plan to answer toxicological, engineering or process-related questions.

The samples discussed in this paper were taken as part of the comprehensive assessment test program (EPA 68-02-2197) conducted by TRW at the coal- and oil-fired industrial boiler at the Firestone Tire and Rubber (Pottstown, Pennsylvania) plant and a coal-fired power plant. Both sites had FGD systems. The Firestone boiler was equipped with a prototype 10 MW dual-alkali system, where the power plant had a full scale (100 MW) commercial limestone system. At both sites, Method 5, SASS and impactors were used to sample the flue gas streams. These samples in turn were analyzed by both Level 1 and Level 2 procefures. This paper discusses selected results from the test program to illustrate the use of state-of-the-art analytical techniques. As a result of these analyses, data was obtained on such topics as SMSS accuracy, trace element enrichment across an FGD, particle size and elemental distribution, surface composition and compound identification.

TECHNIQUES FOR ELEMENTAL ANALYSIS

Acquiring basic information on elemental emission concentrations from combustion sources is becoming a major part of any site characterization. Methods of analysis must be able to meet the Level 2 accuracy and sensitivity requirements while being cost effective. The dependable Atomic Absorption Spectroscopy (AAS) is, and will be, the mainstay of future elemental analysis. However, for more cost effective analyses, multi-element techniques will be required. Classical techniques like DC or AC arc emission are still used to provide generally qualitative elemental information for specific matrices. The Industrial Environmental Research Laboratory at RTP has recommended Spark Source Mass Spectrometry (SSMS) as its Level 1 elemental analysis method. While it is sensitive (ppb) and has a wide elemental range, its accuracy as employed in the Level 1 (1) procedures is approximately a factor of 2-3. Unfortunately, uncertainties in SSMS data can be much greater than those quoted limits. Figure 1 shows a histogram of the ratio of SSMS to AAS values for 17 elements (Aℓ, As, Ca, Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, Pb, Sb, Se, Si, V, Zn), analyzed by SSMS and graphite rod atomizer AAS. The samples were obtained at the inlet and the outlet of the coal-fired power plant limestone wet scrubber.

When SSMS had a "greater than" number, the upper limit listed was used to determine the ratio to the AAS value (Δ). Real number to real number comparisons are shown as (0). Solid circles or triangles are data from the inlet while the open circles or triangles represent data from the outlet gas stream samples. While in the qualitative sense the SSMS data fulfilled its role, the quantitative comparison was not as good. Overall, only 55% of the comparisons were within a factor of 3. Real number comparisons showed that 85% of the SSMS values were within a factor of 3. Inlet SSMS results were within a factor of three 69% of the time compared to 44% for outlet values.

Comparison of the 17 elements analyzed by both methods shows good agreement (values within a factor of two) only for inlet and outlet concentrations of Co, Ni, Sb, Se, and V. Order of magnitude agreement was obtained for Ca, Cd, Be, Fe, Mn, Pb, Sr, and Zn. SSMS analysis of other elements, such as Aℓ, As, Cu and Cr, correspond to the AAS values only to within approximately two orders of magnitude.

Within the last 5 years a new method, Inductively Coupled Plasma Optical Emission Spectroscopy, has attracted a lot of attention by commercial manufacturers and the EPA. ICAP, the most commonly used abbreviation for Inductively Coupled Argon Plasma, is essentially a flame emission technique where atoms are thermally excited and emit light at discrete wavelengths. The atomization and excitation source is an argon plasma torch whose temperature is on the order of 9000°K. In practice, samples in the form of a liquid are aspirated into the argon plasma torch, and the emitted light is separated by a curved grating and quantitated by preset photomultiplier tubes. Figure 2 shows a block diagram of a plasma torch and spectrometer.

Current commercial instrumentation employs up to 60 photomultiplier tubes so that 60 preselected elements can be measured simultaneously. For the quantitative analysis, standard solutions and calibration curves have

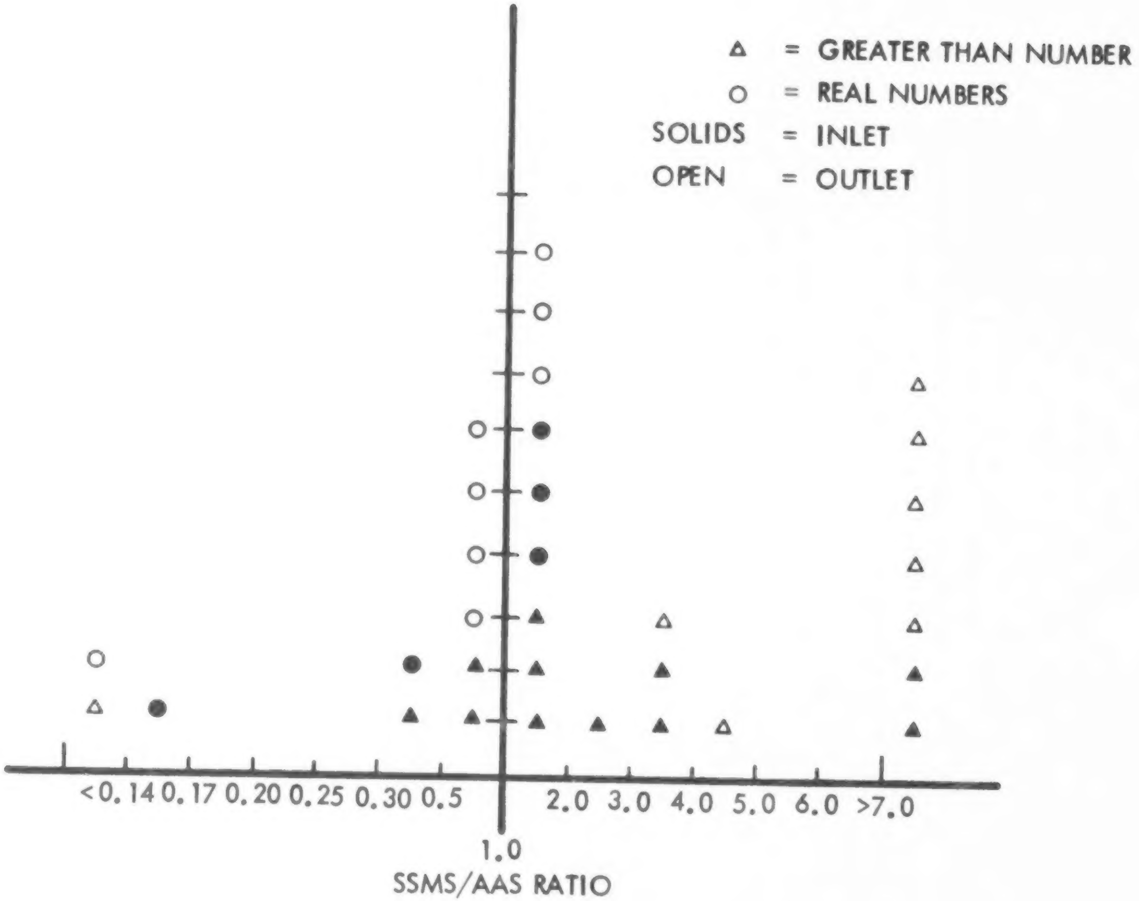

FIGURE 1 SSMS/AAS comparison histogram of the ratio of SSMS to AAS for seventeen elements.

to be made. Since ICAP is a solution technique, solids have to be dissolved in acid and/or undergo a fusion and acid digestion to solubilize them.

ICAP has a limit of detection on the order of 10^{-2} $\mu g\ ml^{-1}$. Table 1 lists some detection limits reported for some elements by ICAP compared to flame and furnace AAS. Sensitivity limits compare favorably with AAS flame but are higher than furnace AAS. However ICAP offers three orders of magnitude linear range for most elements, and the advantage of routine analysis for 30-40 elements simultaneously.

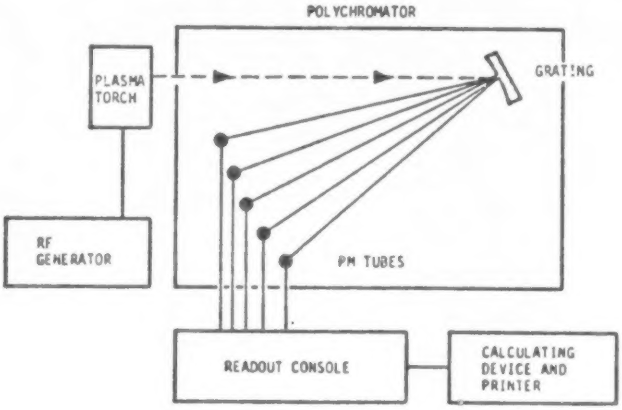

FIGURE 2 A block diagram of a plasma torch and spectrometer

The EPA has extensively evaluated ICAP (3) for use in drinking and wastewater applications and late this summer the EPA proposed ICAP as an alternate method of analysis for effluent guideline work. Besides demonstrating good accuracy and sensitivity, cost per analysis for 40 elements ranges from $30 to $80, depending on the vendor. Vendor availability is good and can be expected to improve as the new generation of less expensive ($60K) ICAP's reach the market.

Illustrative of the use of elemental data from ICAP was the work that TRW performed as part of a comprehensive assessment program sponsored by IERL/RTP (4). During part of this program an evaluation was made of the Firestone Tire and Rubber coal- and oil-burning industrial boiler, which was partially controlled by a prototype double alkali venturi scrubber with a cyclonic mist elimination FGD system. The test program included sampling at the inlet and outlet gas streams of the scrubber as well as selected liquid or slurry streams. Both coal and oil firings were conducted so that a comparison of the fuels could be made.

The elemental data obtained from the gas sampling tests showed that all of the trace elements studied were removed to some degree, as would be expected from the mass removal data which showed 99% and 84% efficiency, respectively, for coal and oil firings.

TABLE 1

Comparative Detection Limits (ppb) (μg/L) (2)

Element	ICP	Flame AA	Furnace AA
Aℓ	10	20	0.004
As	15	100	0.060
B	2	1000	
Cd	1	1	0.008
Co	2	5	0.03
Cr	2	3	0.005
Cu	2	2	0.008
Fe	1	5	0.003
Mn	0.5	3	0.004
Mo	5	10	0.06
Ni	5	8	0.02
P	30	10^5	3.0
Pb	15	10	0.03
Pt	20	50	0.45
Se	15	100	0.10
Si	10	60	0.10
Ti	1	50	0.30
U	75	7000	
V	2	20	0.15
Zn	1	0.6	0.0007

We were interested to determine whether the scrubber affected the composition of the particulate matter. To study this question the individual elemental concentrations (ng/J) were ratioed to a selected element. The element selected was aluminum since it was part of the basic fly ash matrix and would not be expected to be partitioned among the different particle sizes. The ratio of an element to aluminum was termed a concentration factor. The next step was to ratio the outlet concentration factor to the inlet concentration factor (enrichment ratio). An enrichment ratio (ER) greater than one indicated that the outlet particulate matter was enriched with respect to that element while an ER < 1 indicated a depletion of that element in the matrix. The ER's were converted into percentage enrichment [(ER-1)100%] and displayed as a histogram (Figures 3 and 4).

It was found that As, Cr, Mn, Ni, Sb, Se, V, and Zn were enriched in the outlet particulate matter for both the coal and oil firings. Questions arose as to why these elements were enriched and why the overall range of the percentage enrichment was different between the coal-and oil-fired tests.

One clue to these differences was the particle size distribution of the inlet and outlet particulate matter (Table 2). In both cases the majority of particles that were emitted by the scrubber were < 1 μm while the

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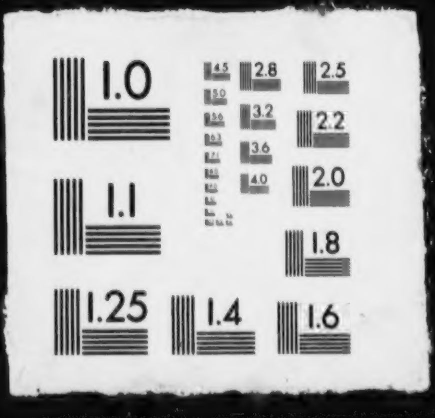
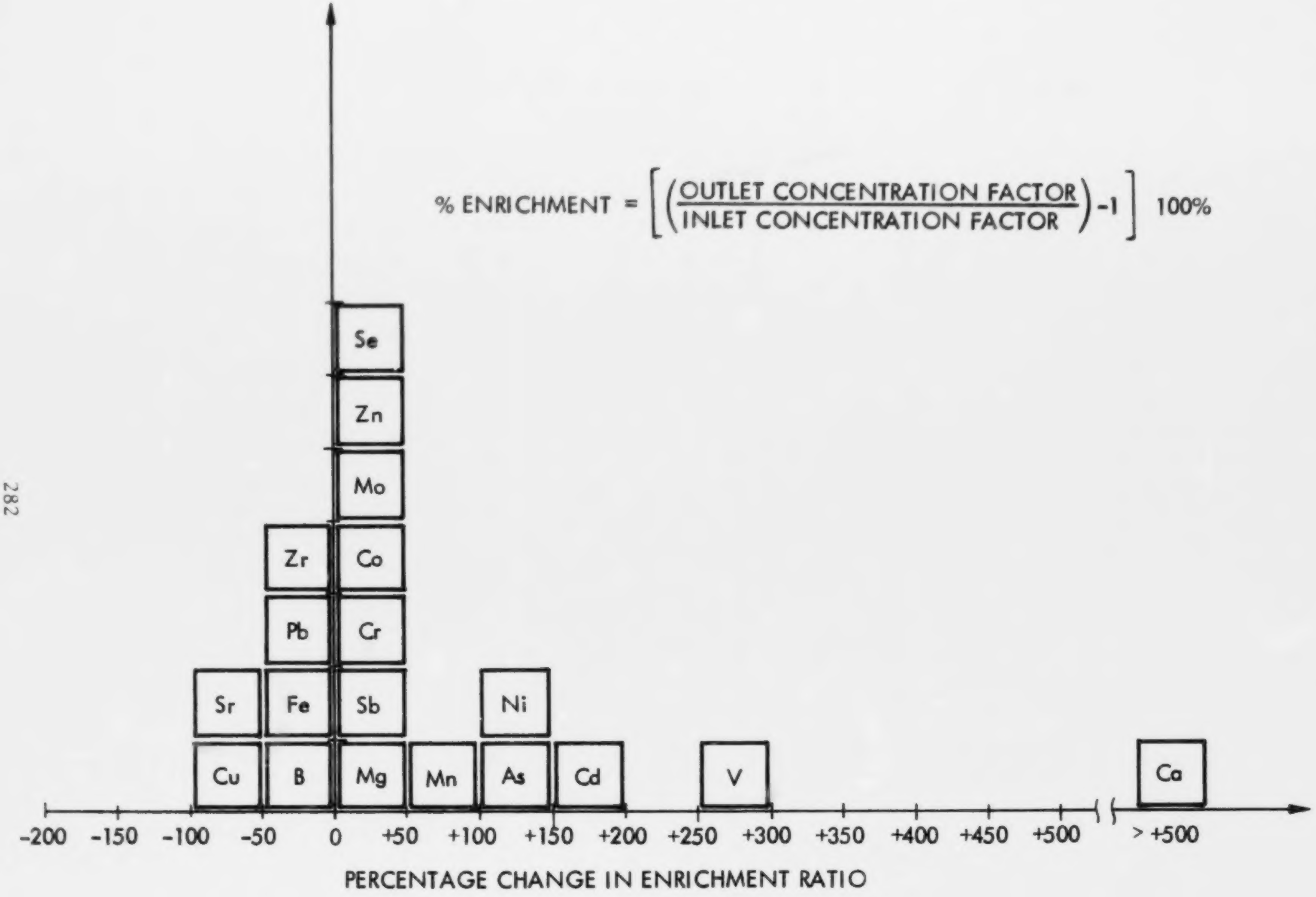

1.0
4.5
2.8
2.5
5.0
5.6
3.2
2.2
6.3
3.6
7.1
2.0
8.0
4.0
1.1
1.8
1.25
1.4
1.6



$$\% \text{ ENRICHMENT} = \left[\left(\frac{\text{OUTLET CONCENTRATION FACTOR}}{\text{INLET CONCENTRATION FACTOR}} \right) -1 \right] 100\%$$

FIGURE 4 Trace element enrichment of outlet particles for oil fired case.

majority of the inlet particles were > 3 μm. The largest difference in the two particle size distributions occurred during the coal-firing where the largest spread in the ER's was also found.

TABLE 2

Scrubber Inlet and Outlet Particulate Size Distribution

Aerodynamic Diameter Size Range, Microns	Weight %			
	Coal Firing (Test 201-1)		Oil Firing (Test 202-1)	
	Scrubber Inlet	Scrubber Outlet	Scrubber Inlet	Scrubber Outlet
< 1	0.0017	62	20	83
1 - 3	0.041	30	1	12
3 - 10	2.24	7	74	5
> 10	97.7	1	5	0

Others (5, 6, 7) have noted these differences when large particles were preferentially removed by a control device. They attributed the enrichment of selected elements to their increased concentration in the smaller particle size. One way to confirm this hypothesis was to collect a sized particle sample and analyze each size fraction. This was what we did at the coal-fired power plant FGD system. In this case, the FGD system was a full sized (∿ 100 MW) limestone scrubber with a venture/absorber combination using Chevron mist eliminators.

A variety of impactors are available for sampling flue gas streams. An MRI 1502 impactor was selected, since the particles could be collected on a greased (Apiezon L) substrate (Kapton film) placed on the collection stage. This substrate with the particles could be removed for elemental analysis without contamination from the 316 SS collection plate. The samples collected in this fashion were analyzed for trace elements using Particle Induced X-Ray Emission (PIXE). In Particle Induced X-Ray Emission the sample is prepared as a thin film and bombarded with a beam of protons or alpha particles which cause the characteristic X-ray emission (fluorescence) from all the elements in the sample. The X-rays are detected by an energy-sensitive semiconductor device and the data are processed by an on-line computer. Figure 5 shows a diagram (8) of the PIXE unit at the Crocker Nuclear Laboratory at the University of California, Davis. PIXE can detect elements Na through U, but the sensitivity (nominally ppm) depends on the element and matrix. In practice a piece of the impactor substrate containing one impaction spot is mounted in a photographic slide and sent for analysis.

Impactor samples were taken at the outlet of the FGD and the impactor data was reduced using the procedure (8,9) developed by Southern Research Institute (SoRI) for IERL/RTP. In this approach $[dM/d(\log D_{50})]$ the data

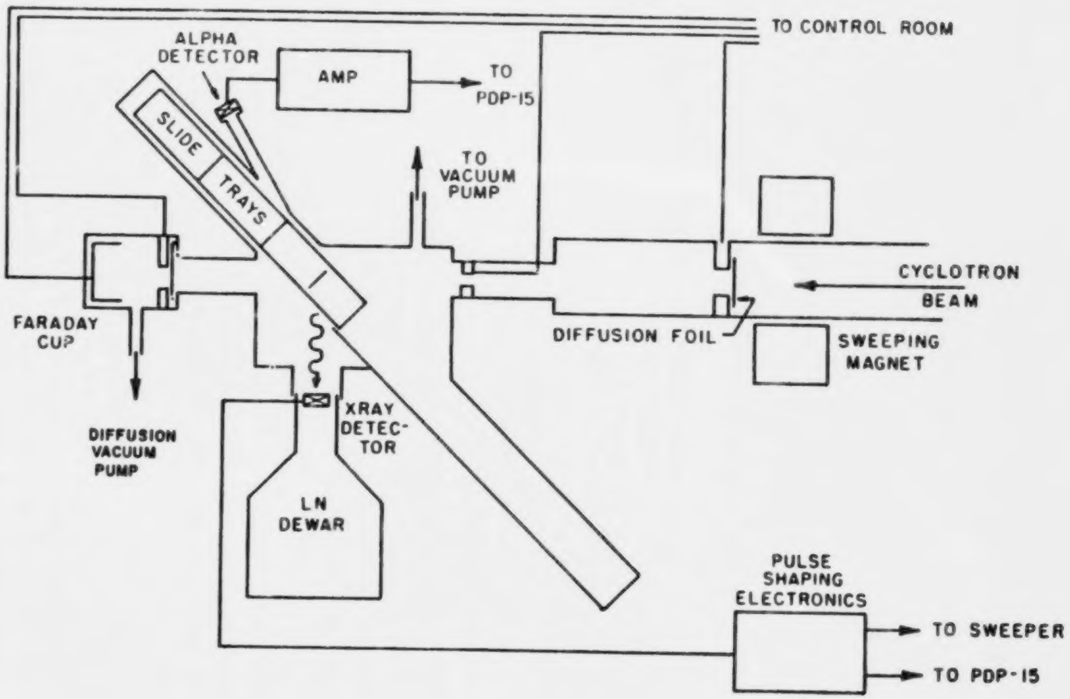

TO CONTROL ROOM
ALPHA DETECTOR
AMP
TO PDP-15
SLIDE
TRAYS
TO VACUUM PUMP
CYCLOTRON BEAM
DIFFUSION FOIL
SWEEPING MAGNET
FARADAY CUP
DIFFUSION VACUUM PUMP
XRAY DETECTOR
LN DEWAR
PULSE SHAPING ELECTRONICS
TO SWEEPER
TO PDP-15

FIGURE 5 University of California, Davis, PIXE system.

is normalized so that a smooth curve can be drawn through the limited number of data points obtained from the impactor. The normalized mass data placed in the dM/d (log D_{50}) format is shown in Figure 6. The data for Run 135 was corrected for a negative filter weight using the weight percentage of the filter from Run 136. The filter data point for 135 is shown in brackets, but even without this correction the data agrees rather well. The slight offset in the cut points is due to the difference in the impactor flow rate during the two runs. In both cases the mass data showed a bimodal distribution with an apparent maximum around 0.4 to 0.5 µm.

In contrast to the mass data, the elemental data obtained from the PIXE analysis showed a maxima at slightly different particle sizes (Figure 7). Other elements were detected, but only the elements with PIXE data for all stages were presented. Calcium, Pb, Zn, and Si had a maximum at 20 µm while S was the only element to show a maximum at 0.5 µm. Iron showed its concentration maximum over the mass minimum. The lower particle size maximum for the elements corresponds closely to the total mass value maximum (0.4 to 0.5 µm). To determine if the elements exhibited a concentration increase with decreasing particle size, the elemental concentrations were ratioed to Si which, because it is a major component of fly ash, should not have exhibited a concentration change over the particle size range. Figure 8 shows the result of this effort. Sulfur showed a consistently increasing concentration with decreasing particle size. The high concentration of sulfur on the filter might be due to H_2SO_4 aerosols being collected since the impactor was run at stack temperatures ($\pm$ 55°C), which would not prevent the collection of H_2SO_4 aerosols. Calcium showed a weak positive trend while Pb and Fe showed a moderately strong trend to increasing concentration at a smaller particle size. Zinc showed the strongest trend of the metals paralleling sulfur throughout impactor stages, but dropping off on the filter.

These data are similar to other researchers (8,9,10) who found that elements like Zn, Pb, and S have increasing concentrations with decreasing particle size. As a result, those elements which tended to concentrate in the smaller particles would show a concentration increase in the outlet particle matter, since the FGD preferentially emits the small particles. This result would be seen as an enrichment ratio greater than one and would be most apparent when the inlet particle distribution is skewed toward the large particles. In the Firestone Coal-firing, as discussed earlier, greater than 95% of the inlet particles were above 3 µm (Table 2). As a result of this reversal of the particle size distribution, the histogram (Figure 3) showed a large increase in the enrichment ratios for selected elements.

The same reasoning would predict that if the inlet particle size distribution was spread evenly or skewed to the smaller (< 3 µm) particles, then enrichment ratios would tend to be near one. In the oil-fired Firestone tests, the inlet particle distribution was weighted toward the smaller particles, and the total mass loading was low. Consequently, removal of the large particles (> 3 µm) did not have as great an effect on the enrichment ratios. This fact was illustrated by Figure 4 which showed that most of the elements exhibited little or no concentration enrichment.

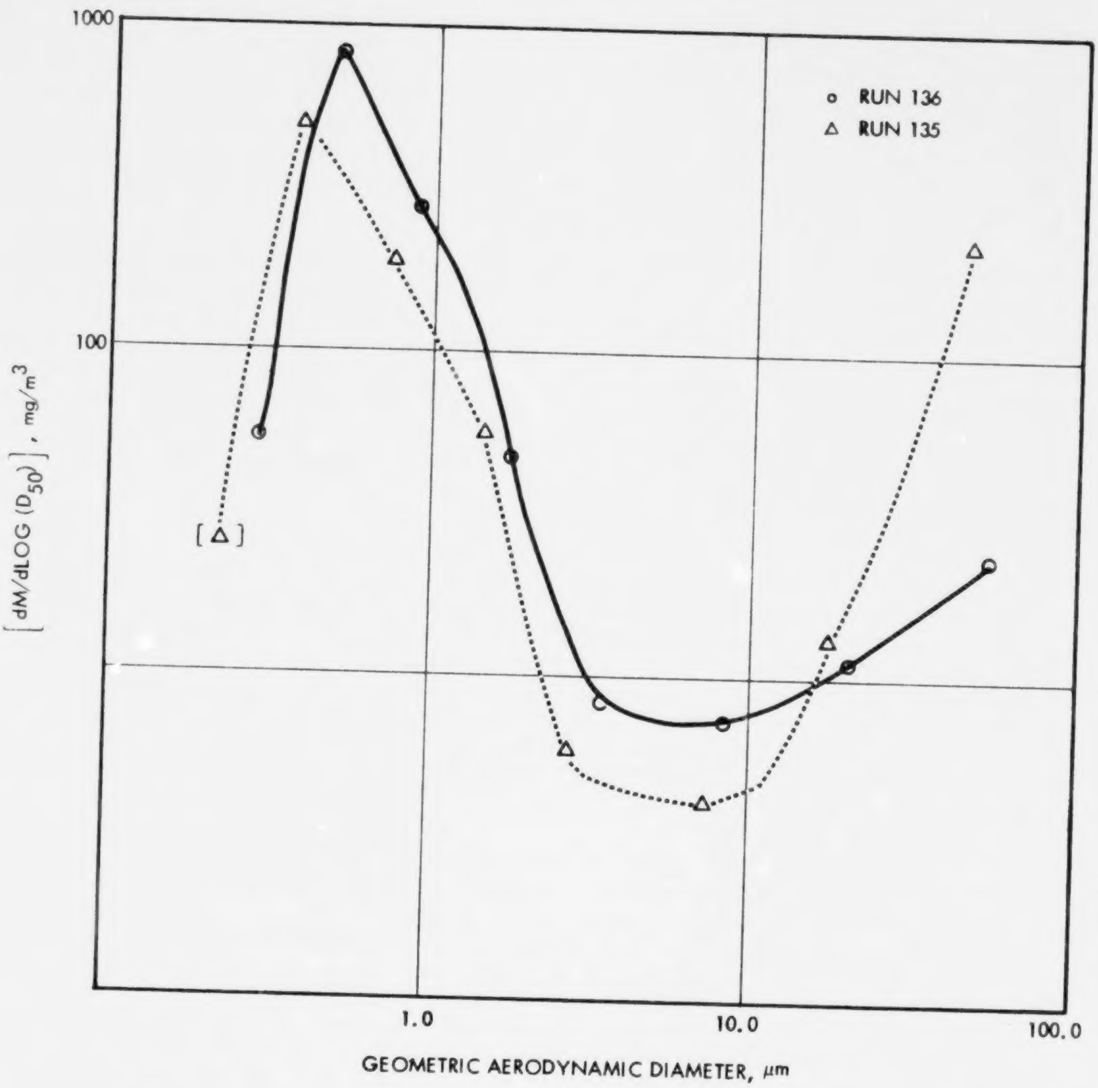


FIGURE 6 Coal-fired power plant outlet impactor study

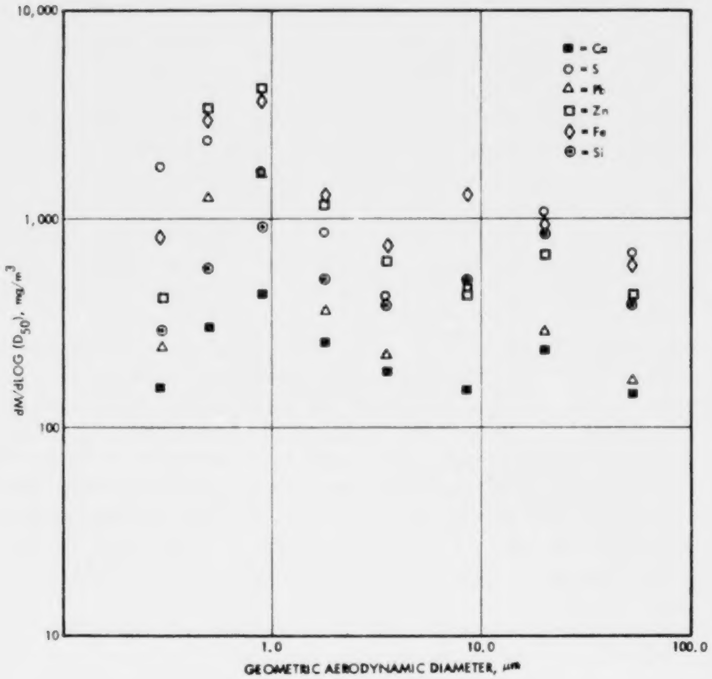


FIGURE 7 Elemental concentration by particle size for fly ash from the outlet of an FGD

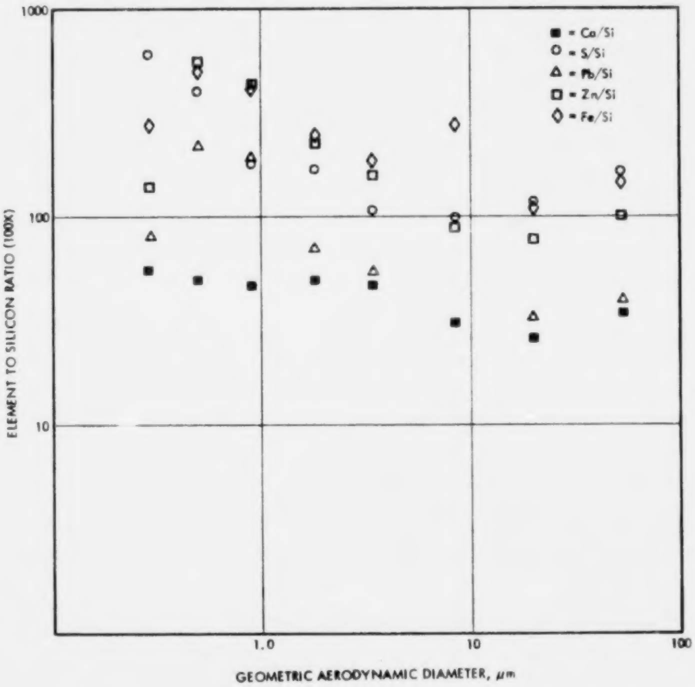


FIGURE 8 Element to Si concentration ratio by particle size

Models have been proposed to explain the apparent concentration of certain trace elements in the smaller particles. Simply stated these models involve the volatilization of elements during combustion, followed by condensation over the magnesium aluminum silicate matrix of the fly ash. (The explanation is not quite this simple - see Kaakinen(5), Smith(6) and Ondov(6) for a more complete explanation). One of the primary tools for surface research is Electron Spectroscopy for Chemical Analysis (ESCA).

In ESCA, low energy X-rays illuminate the surface of the sample. Absorption of these X-rays result in prompt emission of photoelectrons from atomic orbitals whose binding energy is less than the energy of X-ray photons. The total energy of the incident X-ray photon to the first approximation is equal to the energy required to remove the electron from the atom (binding energy). Additional energy is required for the recoil energy of the electron and for the work function of the spectrometer, but both represent less than 1% of the incident energy. Energy in excess of the binding energy appears as the kinetic energy of the electron when it leaves the atom, and is measured by an electron spectrometer. Subtracting the kinetic energy of the electron from the energy of the incident X-ray leaves the electron binding energy, which is only slightly modified by the work function. In commercial electron spectrometers, this subtraction is carried out electronically so that a spectrum of binding energy versus electron counts is produced. Figure 9 shows a typical ESCA spectrum taken of fly ash collected at the outlet of a Fluidized Bed Combustor (FBC).

ESCA is a surface sensitive technique because the photoelectrons cannot travel great distances in solids without undergoing scattering and loss of energy. It is only those which originate near the surface that leave the sample with their full compliment of energy and appear as part of a peak in the ESCA spectrum. Escape depth varies as a function of electron kinetic energy and the type of material being observed. It is normally in a range 5-15 Å for metals, 15-20 Å for inorganic compounds, and 50-100 Å for organic materials. Combining this surface analysis capability with Ar^+ sputtering allows the chemist to remove individual layers and analyze successive surface elemental concentrations.

Chemical information available in ESCA spectra beyond the elemental composition is due to the "chemical shift" effect. Metals exhibit a shift to higher binding energies as they move to higher oxidation states. In this fashion high resolution ESCA can tell the difference between species like C or $CO_3^=$ and $S^=$, $SO_3^=$ and $SO_4^=$. Figure 10 is an actual high resolution ESCA spectrum at 100 Å depth of 27 μm particles from the Battelle FBC unit. The surface spectrum only showed S^{+6}, but after sputtering, a clear layer or particle of S^{-2} was uncovered. The surface layer of pure $SO_4^=$ was probably from the air oxidation of the sulfide compound.

To illustrate the depth profile capability of ESCA, Figures 11 and 12 summarize the ESCA analysis performed on particulate samples from the La Cygne power plant tests. The samples were obtained from the inlet and outlet of

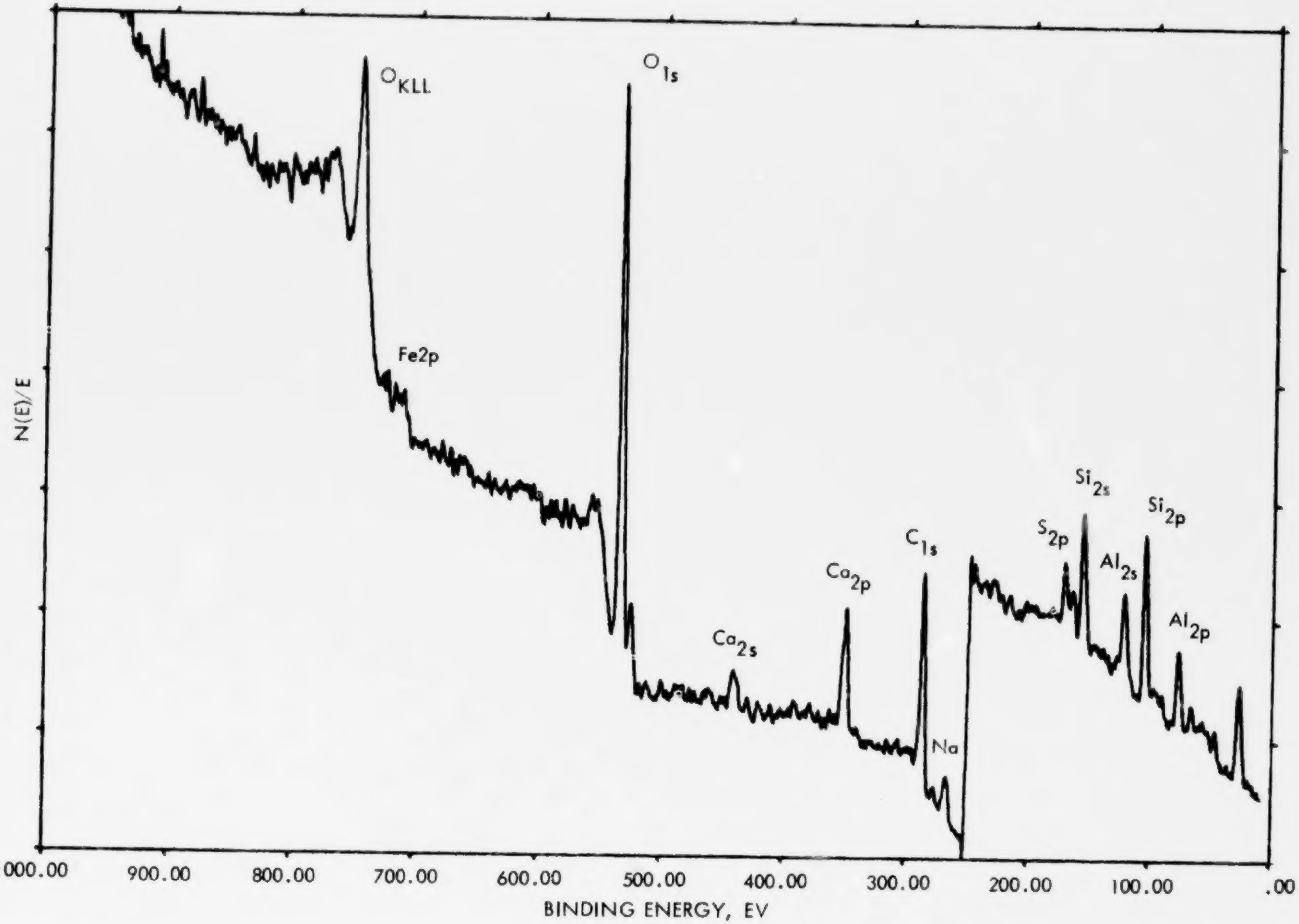


FIGURE 9 Survey ESCA spectrum of fluidized bed combustor sample

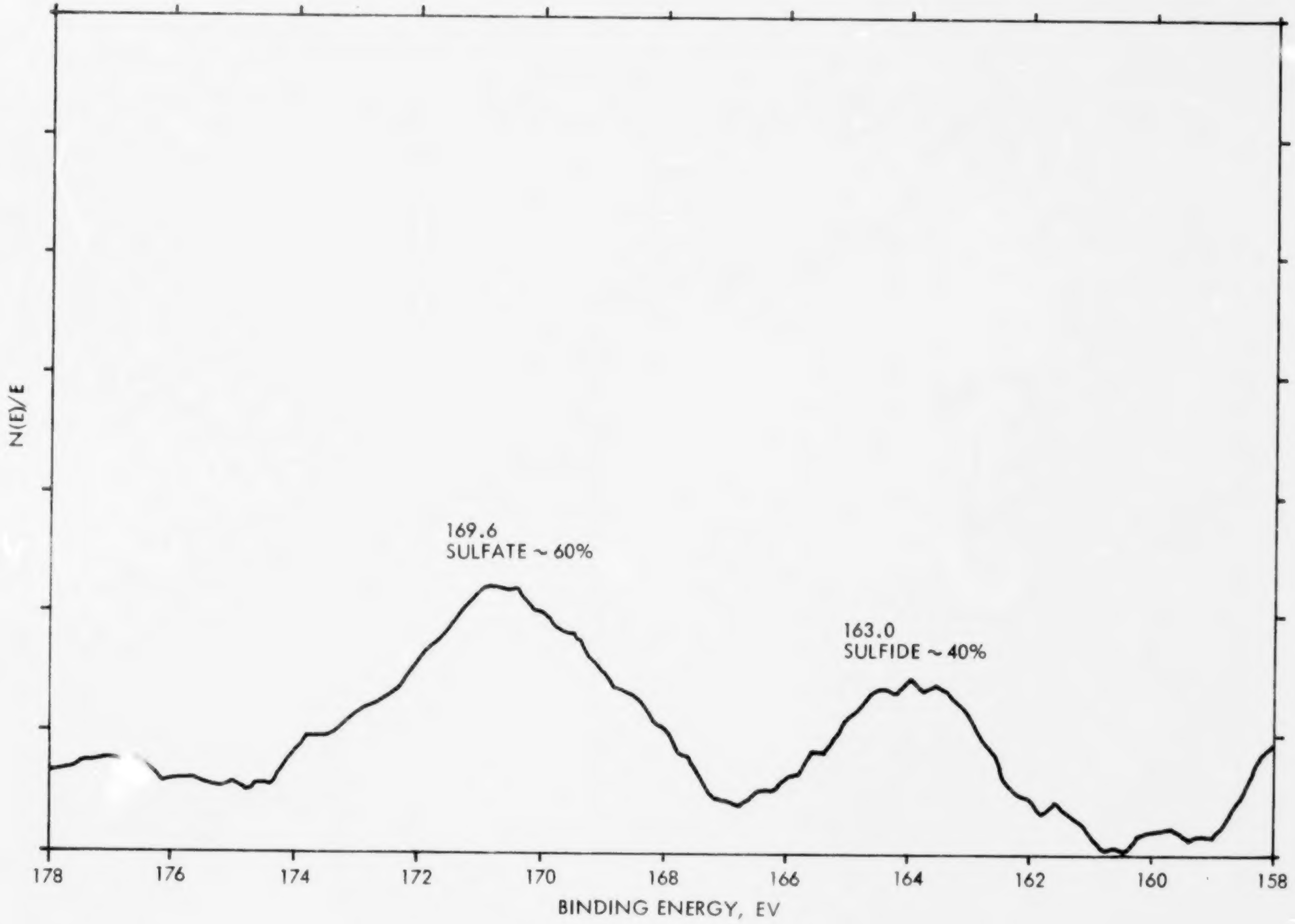


FIGURE 10 High resolution ESCA spectrum of sulfur compounds in a fluidized bed combustor fly ash sample

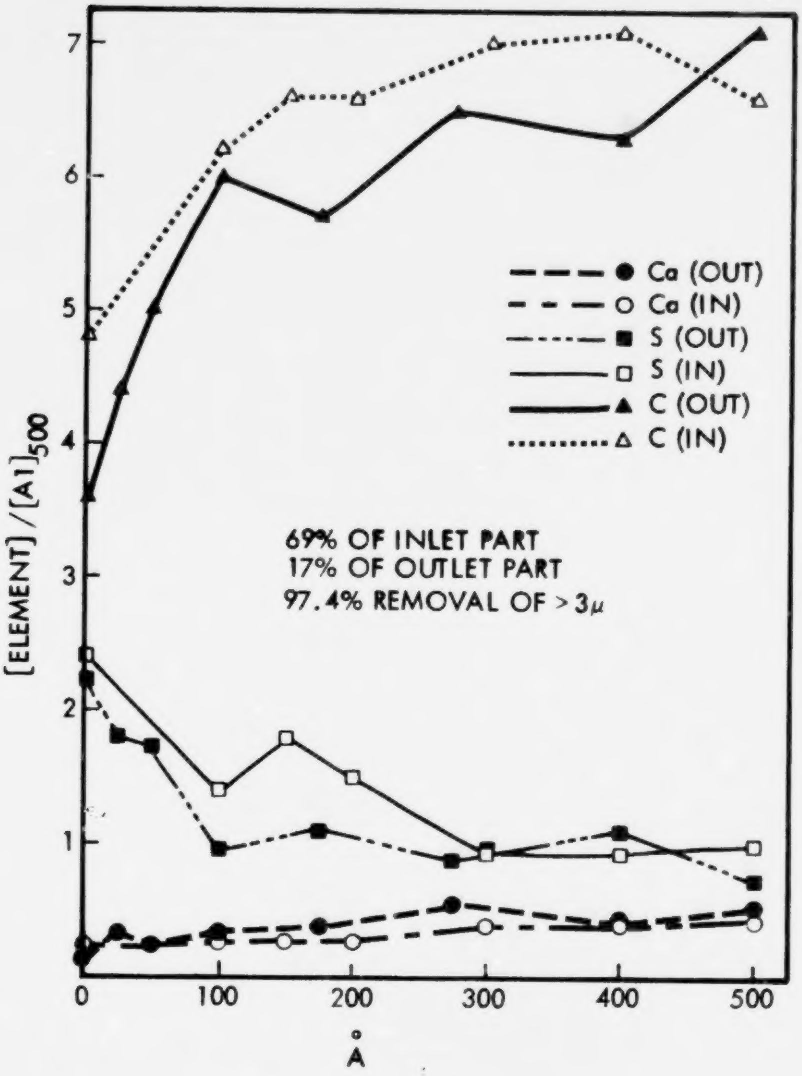


FIGURE 11 ESCA depth profile analysis of ≥ 3 μm particulate matter before and after an FGD

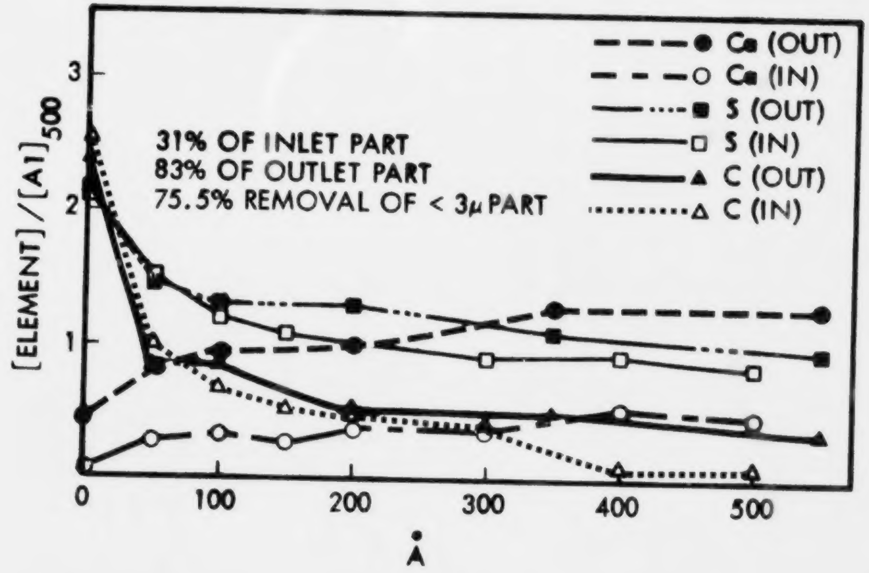


FIGURE 12 ESCA depth profile analysis of <3 μm particulate matter before and after FGD

the limestone FGD using an EPA Method 5 train with a cyclone and filter. The data for Ca, C, and S are presented as atom percentages, normalized to 100%, and ratioed to the Aℓ concentration of 500 Å. These are relative values, not absolute concentrations, since not all elements present in the particles are included. Ratioing the elemental concentrations to the Aℓ concentration at 500 Å accentuates any surface concentration and tends to normalize any analysis errors.

Relative concentrations for the Method 5 cyclone (particles >3 μm) showed that the sulfur/aluminum ratios for both the inlet and outlet particles were high at the particle surface and decreased with depth. This type of data would be expected if a volatile compound of S were adsorbed or condensed on the particles. Note that the sulfur concentration tended to level out above zero, indicating that the coated particle contained sulfur. Later XRD analysis confirmed the presence of $CaSO_4$ in the inlet and outlet cyclone catches. The sulfur rich surface layer was probably formed by H_2SO_4 condensing on the particles. An opposite effect was seen for carbon; the C/Aℓ ratio for the cyclone samples increased with depth, which might indicate that unburnt coal particles were present in the inlet and outlet >3 μm particles. The Ca/Aℓ for the inlet and outlet cyclone and inlet filter samples were very similar, both showed a slight depletion at the surface and roughly equivalent concentrations. The outlet filter showed a higher concentration of Ca, which, after 100 Å, corresponded nicely with the S concentration.

The relative carbon concentration of the inlet filter (<3 μm) particles was much lower than the cyclone fraction and showed a sharp surface dependence. Interestingly, the C/Aℓ ratio went to zero after 400 Å for the inlet samples, but in the outlet filter samples remained fairly constant after the initial drop, which might indicate the presence of carbon containing particles. In this case it is possible that <3 μm particles of unburnt coal (concentrated in the outlet filter by removal of the large particles by the FGD) or $CaCO_3$ (a scrubber reaction product) might be present. The S/Aℓ ratio indicated a surface layer of sulfur rich material similar to that found in the cyclone catch.

The impactor samples from the outlet of the FGD system at the La Cygne were also analyzed by ESCA to determine the relative surface and depth profile concentrations as a function of particle size. An interesting relationship was found when the Ca/S ratio was computed by particle size and at various depths (Table 3). The first two stages exhibited mixed trends that might be due to a mixture of fly ash and a Ca-S compound coated with H_2SO_4. The particles in the 8.5 and 3.5 μm size fraction appear to be primarily a Ca-S compound with little or no coating of H_2SO_4. The particles in the 1.8 μm fraction exhibits a medium coating of H_2SO_4 on a Ca-S particle. Finally the last three stages show a thick coating of H_2SO_4 and may represent H_2SO_4 droplets formed when the H_2SO_4 vapor entering the FGD was rapidly cooled in a stream of fine particles. This interpretation would also explain the PIXE data which showed increasing sulfur concentrations with decreasing particle size (Figure 8).

TABLE 3

ESCA Data - S/Ca Ratio by Particle Size (μm) and Depth (Å)

Depth (Å)	Average Geometric Aerodynamic Diameter (μm)							
	53.9	20.2	8.5	3.5	1.8	0.9	0.51	0.29
Surface	2.78	3.23	–	1.35	5.56	7.69	–	–
100-500	4.55	2.50	0.83	1.28	1.52	7.14	6.25	11.11
500	–	–	1.16	1.00	0.93	4.76	5.00	2.94

(–) ESCA spectra not run

COMPOUND IDENTIFICATION PROCEDURES

It is possible that future emission regulations might control specific elements rather than gross physical parameters like TSP. The level at which these elements will be regulated will be selected in part by the toxicity of the element emitted. Because the specific compound in which the element resides affects the toxicity of the element, information on the type of compound being emitted must be included along with the total output of the element.

While the inorganic analytical chemist has a variety of sources of information, he does not have a universal identification technique like GC/MS. Using accurate elemental and anion concentrations, a list of candidate compounds can be developed to reduce the number of potential compounds and estimate the expected concentrations. Prior knowledge of the expected concentration for a specific compound will aid in the selection of the appropriate identification technique. The primary compound identification techniques for the inorganic analytical chemist are IR spectroscopy and X-ray Diffraction (XRD). Infrared spectroscopy has been a traditional supplement to XRD for inorganic compound identifications. Since XRD can only identify crystalline materials, IR spectroscopy provides information on many compounds which cannot be seen by XRD due to their amorphous nature.

Within the last ten years a revolution in IR spectroscopy has occurred with the advent of Fourier Transform Infrared (FTIR) instruments (11). Both conventional and FTIR spectrometers produce a spectrum of intensity versus wavelength, but there are drastic differences in how the spectrum are produced. The key component of a conventional IR is a monochromator which sorts the polychromatic radiation into discrete bundles of nearly monochromatic radiation. In FTIR a monochromator is replaced by a Michelson interferometer (shown in Figure 13). The light beam is split and falls on both a moving and stationary mirror. When the two beams are recombined, they will either constructively or destructively interfere with each other since a path difference has been added to one beam. The signal generated will be a cosine wave, whose strength will be the sum of all the cosine oscillations caused by the optical frequencies in the source. The interferogram (Figure 14) generated is related back to the original spectrum by a cosine Fourier transform pair. In practice this is a complex operation and requires a dedicated minicomputer.

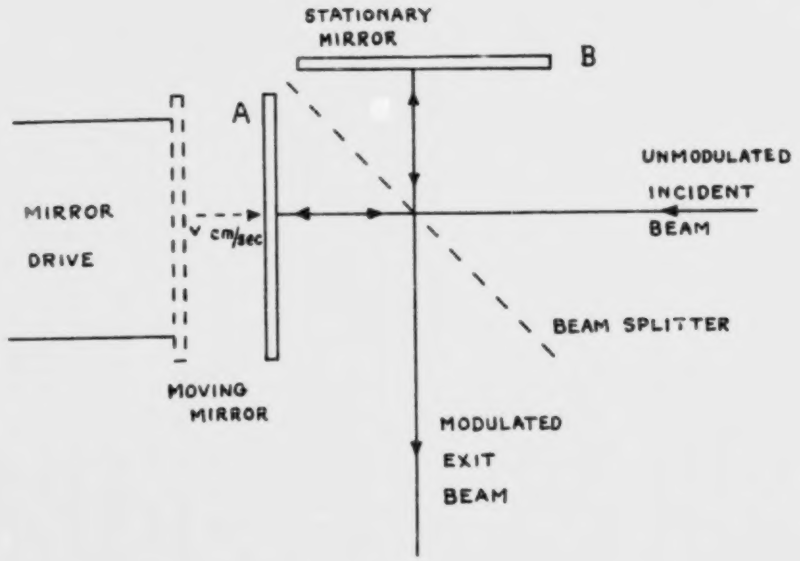

STATIONARY MIRROR
B
A
UNMODULATED INCIDENT BEAM
MIRROR DRIVE
v cm/sec
BEAM SPLITTER
MOVING MIRROR
MODULATED EXIT BEAM

FIGURE 13 Diagram of Michelson Interferometer

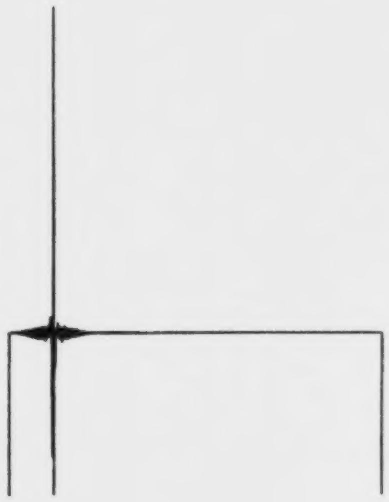


FIGURE 14 Interferogram

The advantages (12) of FTIR outweigh its initial high cost through improved sensitivity, resolution, and data manipulation. Improved sensitivity is obtained as a direct result of the increased energy throughput (Jacqunot's advantage) available in an FTIR due to the elimination of slits. An interferometer has a circular aperture of 50 mm in diameter compared to slit area of $\sim$1 mm^2 for conventional IR.

In FTIR a complete spectrum is taken in approximately 1 second which is about the time it takes for a conventional IR to scan one wavelength. From this, one can see that for equivalent experiments the FTIR system is N times faster than a dispersive spectrometer (where N is the number of resolution elements in the spectrum) or that the FTIR has $N^{1/2}$ more analytical sensitivity than the despersive spectrometer for the same measurement time. This multiplex or Fellgett advantage can be exploited by co-adding successive interferograms to increase the signal-to-noise ratio. Since the signal increases with the number scans and the noise with the square root of the number of scans, the signal-to-noise ratio increases with the square root of the number of scans.

The most important advantage for FTIR is that the spectrum is present in a digital form and can be processed easily using the minicomputer. In this way, spectra can be co-added, subtracted, ratioed, or stored for future use. This allows a comparison of different spectra and permits small differences in the spectra to be easily detected.

When sampling at the outlet of an FGD most often the mass loading is so low that the total particulate mass collected is <200 mg. This small amount of material is also impacted onto the glassfiber filter making sample removal for analysis next to impossible. TRW has developed FTIR procedures to circumvent these problems.

The general approach was to run each filter sample, standards on filters, and blank filters using an Attenuated Total Reflectance (ATR) cell. The standards were prepared using the apparatus in Figure 15. A known amount of the standard was placed at the bottom of the jar and a pre-weighed filter ($\sim$8 cm dia.) was loaded into the open faced filter holder. After the apparatus was assembled, the vacuum pump was turned on to initiate the air flow and to entrain the particles of the standard material. After a period of time, the filter was removed and re-weighed. The mg/cm^2 loading was determined and a 10 x 50 mm section was removed from the filter for FTIR-ATR analysis. Figure 16 shows the FTIR spectra obtained for a $CaSO_4$ standard made in this fashion. The broad bands at 975,, 775 and 450 cm^{-1} are due to the glassfiber filter and would interfere with later interpretations. Using the subtraction capability of FTIR, the blank glassfiber filter spectra was subtracted from the standard spectra. The resulting spectra (Figure 17) is equivalent to standard reference spectra for $CaSO_4$, yet, having been run as the samples would be, it reflects any anomalies introduced by the ATR procedure. A series of spectra were run for $CaSO_4$, $CaSO_3$ and H_2SO_4 at various surface loadings and stored in the FTIR's computer for later spectral interpretation.

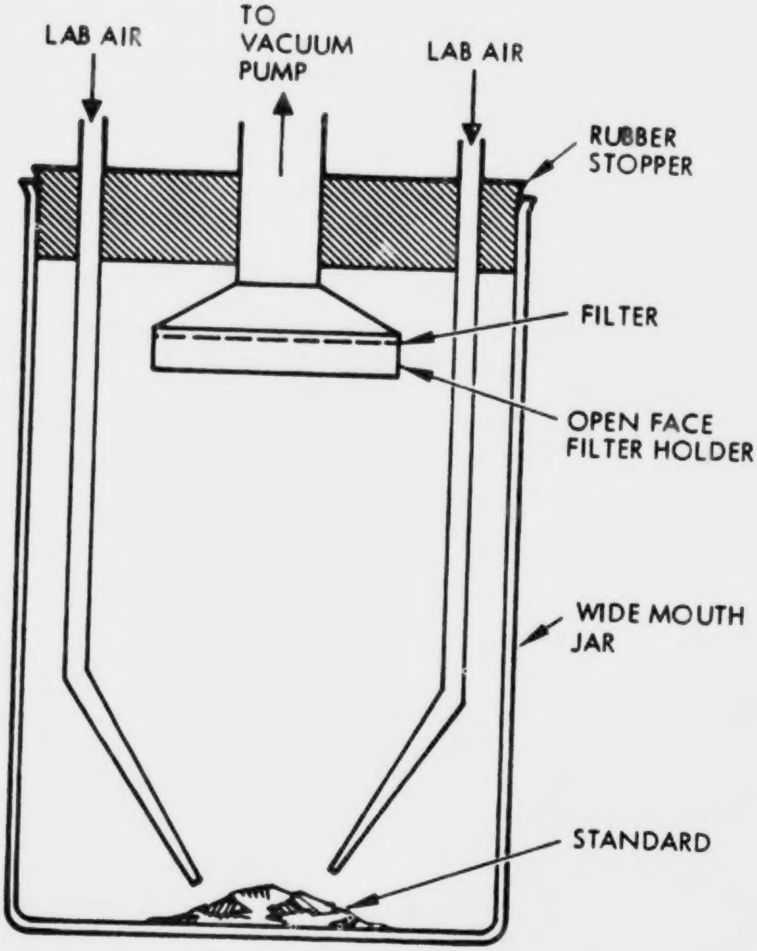

LAB AIR
TO VACUUM PUMP
LAB AIR
RUBBER STOPPER
FILTER
OPEN FACE FILTER HOLDER
WIDE MOUTH JAR
STANDARD

FIGURE 15 Powder dispersion apparatus used to prepare FTIR standards on glassfiber filters

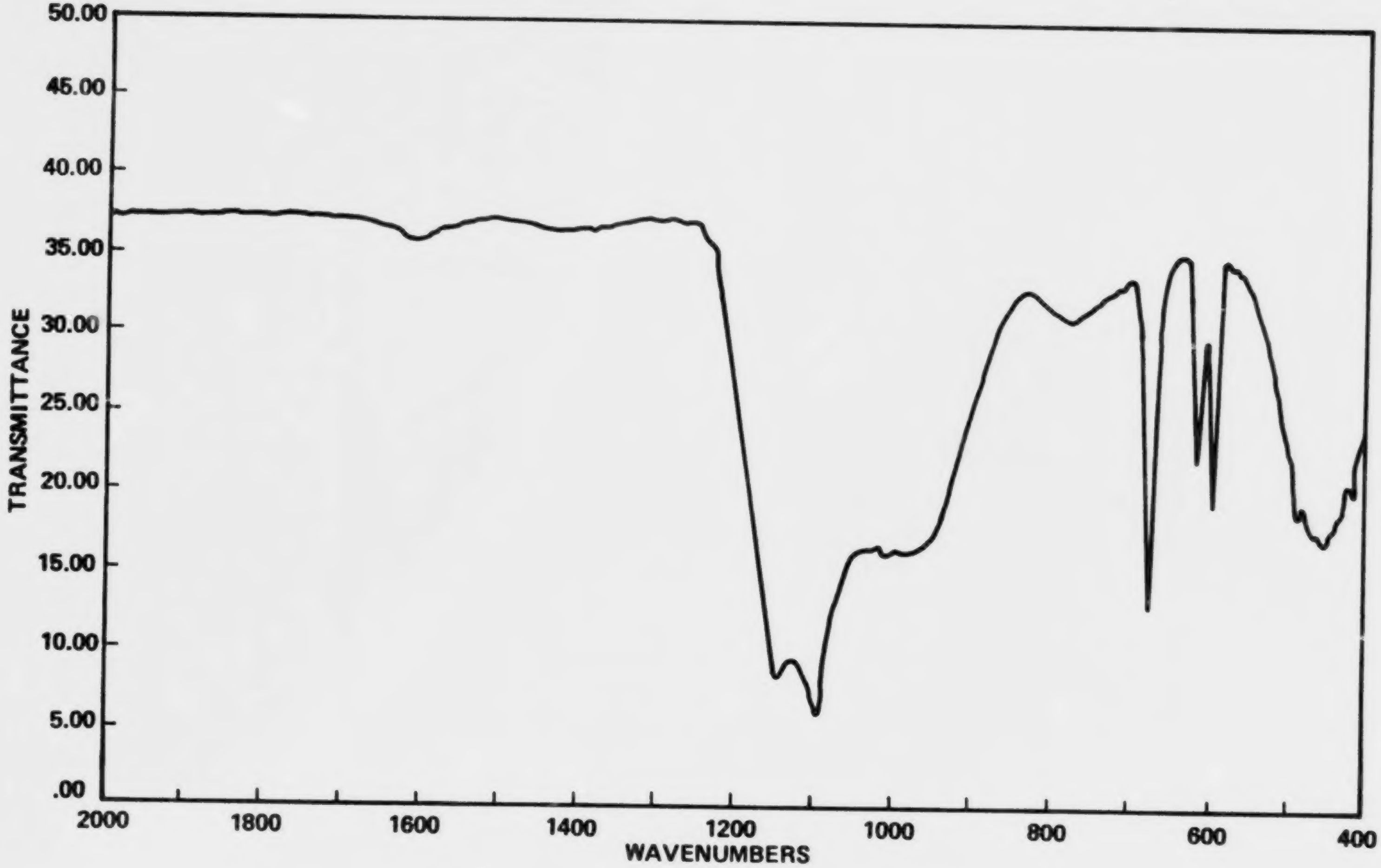


FIGURE 16 FTIR spectrum of $CaSO_4$ standard on glass fiber filter

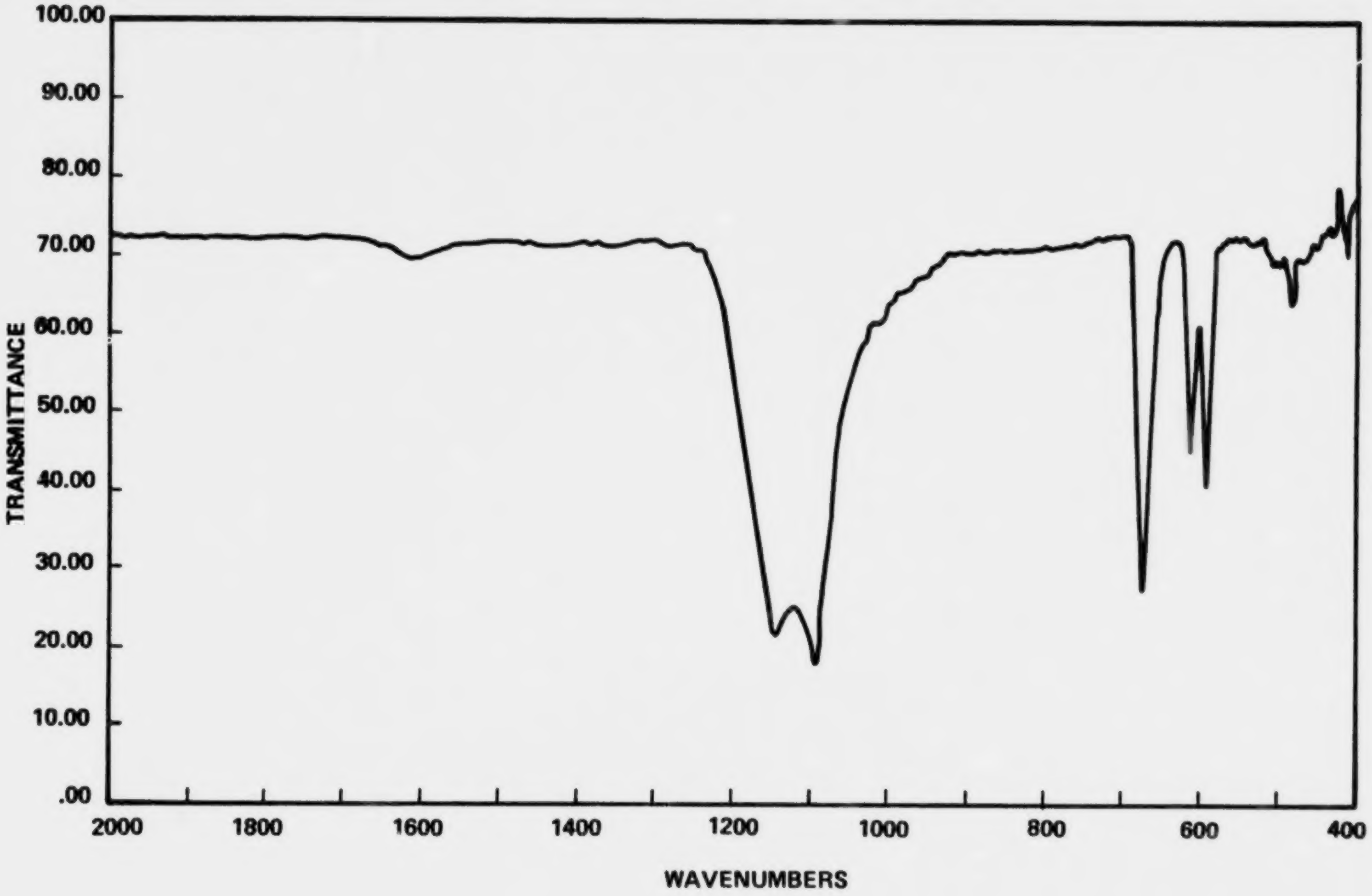


FIGURE 17 FTIR spectrum of $CaSO_4$ standard with glass fiber filter gackground removed

Sections of filter samples taken at the inlet and outlet of a limestone FGD were run and corrected for the glassfiber filter background. Since we were interested in the differences between the inlet and outlet particulate matter, the inlet FTIR filter spectra was subtracted from the outlet filter spectra. The original inlet and outlet spectra as well as the difference spectra are displayed in Figure 18. Any bands which appear in the outlet spectra are either unique to the outlet spectra or represent compounds which are present at higher levels in the outlet particulate matter. Once the difference spectra was generated, known percentages of the standard's ($CaSO_4$, $CaSO_3 \cdot 1/2H_2O$ and H_2SO_4) spectra were added to the difference spectra. By electronically "spiking" the difference spectra with the standard, unknown compounds were identified by noting which bands increased in absorbance. For example, Figure 19 shows the difference spectrum and the addition spectrum after 3% of the 0.44 mg/cm^2 $CaSO_4$ standard was added. Note the increased absorbances at 1150, 1095, 670 and 620 cm^{-1} and compare those bands to the original difference spectrum. Clearly $CaSO_4$ was present. After the identification of $CaSO_4$ in the outlet material, the next step was to quantitate the amount of $CaSO_4$ in the filter.

The original idea was to use the different surface loading standards to develop a calibration curve. It was found that the extention coefficient decreased with increasing surface loading. At first it was thought that the amount of $CaSO_4$ in each filter was incorrect. However, after reviewing the results of some quantitative XRD work, it was decided that the weights were correct. The reason for the change in the extinction coefficient was related to the amount of sample which the FTIR beam sees in an ATR experiment. At low surface loadings nearly a monolayer of sample is present, and since the beam interacts with those particles in contact with the ATR plates, essentially all of the sample was "seen" by the beam. As the surface layers were increased due to increased loading, the actual amount of sample in contact with the ATR plates decreased. Consequently, as the surface loading increases, the extinction coefficient decreases.

In order to circumvent this problem, the difference spectrum was electronically "spiked" with varying (31, 3, 5 10%) amounts of the 0.13 mg/cm^2 and 0.44 mg/cm^2 $CaSO_4$ standard's spectra. A standard additive curve was generated for each of the $CaSO_4$ standard's surface loading.

The results are shown in Figure 20. Clearly, different response factors were encountered, but in the final analysis the calculated values for $CaSO_4$ of 2.3 mg/cm^2 and 3.1 mg/cm^2 were within 15% of each other.

This work with FTIR illustrates the type of qualitative and quantitative experiments which can be performed. Additional work is needed to develop a library of spectra of scrubber reaction products. These spectra would be stored on tape or disc for ready access. In fact this entire approach could be extended to selected hazardous or regulated substances to provide rapid, accurate and sensitive analysis for those compounds in environmental samples.

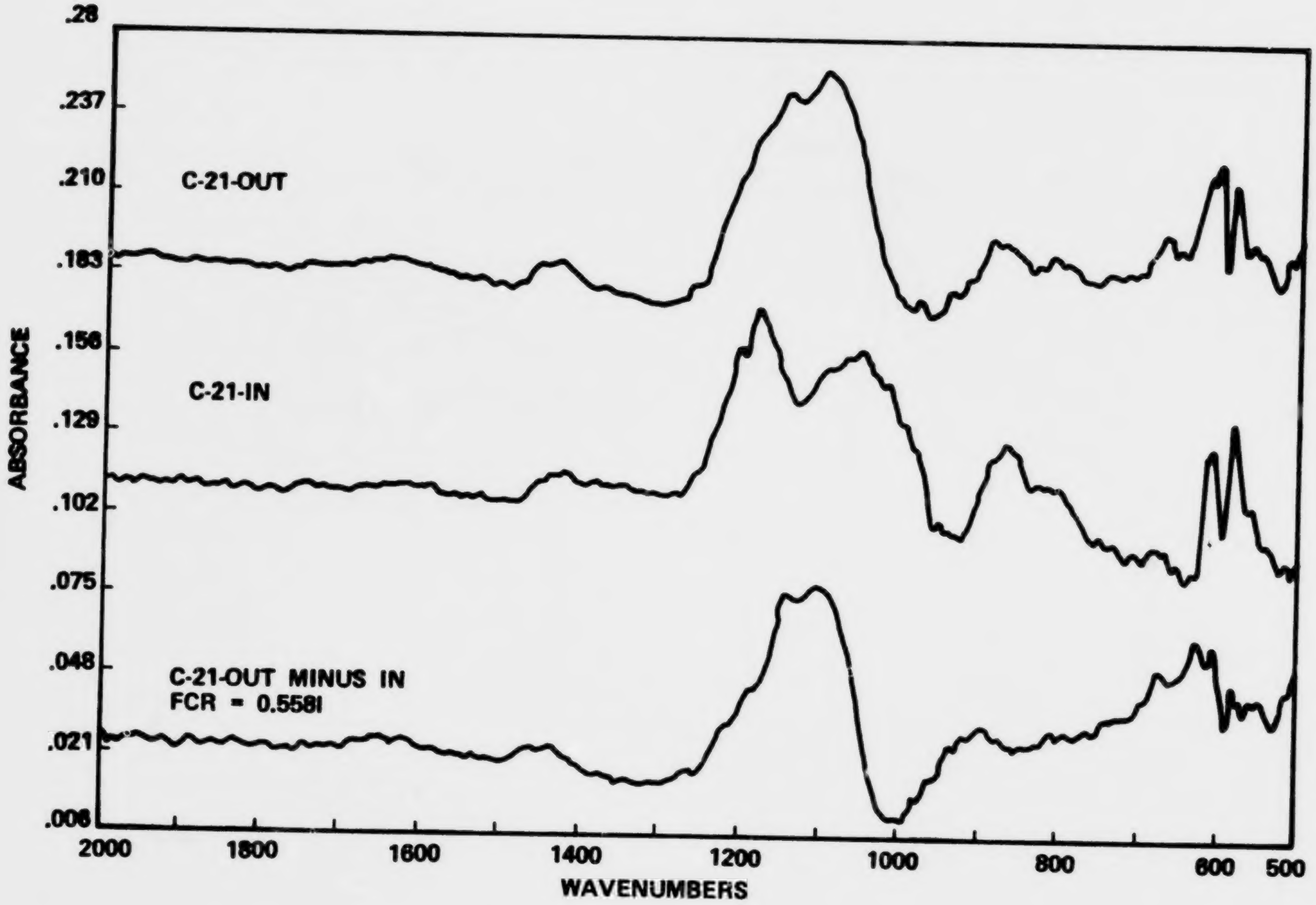


FIGURE 18 FTIR spectra of inlet and outlet FGD particulate matter and their difference spectrum (outlet minus inlet)

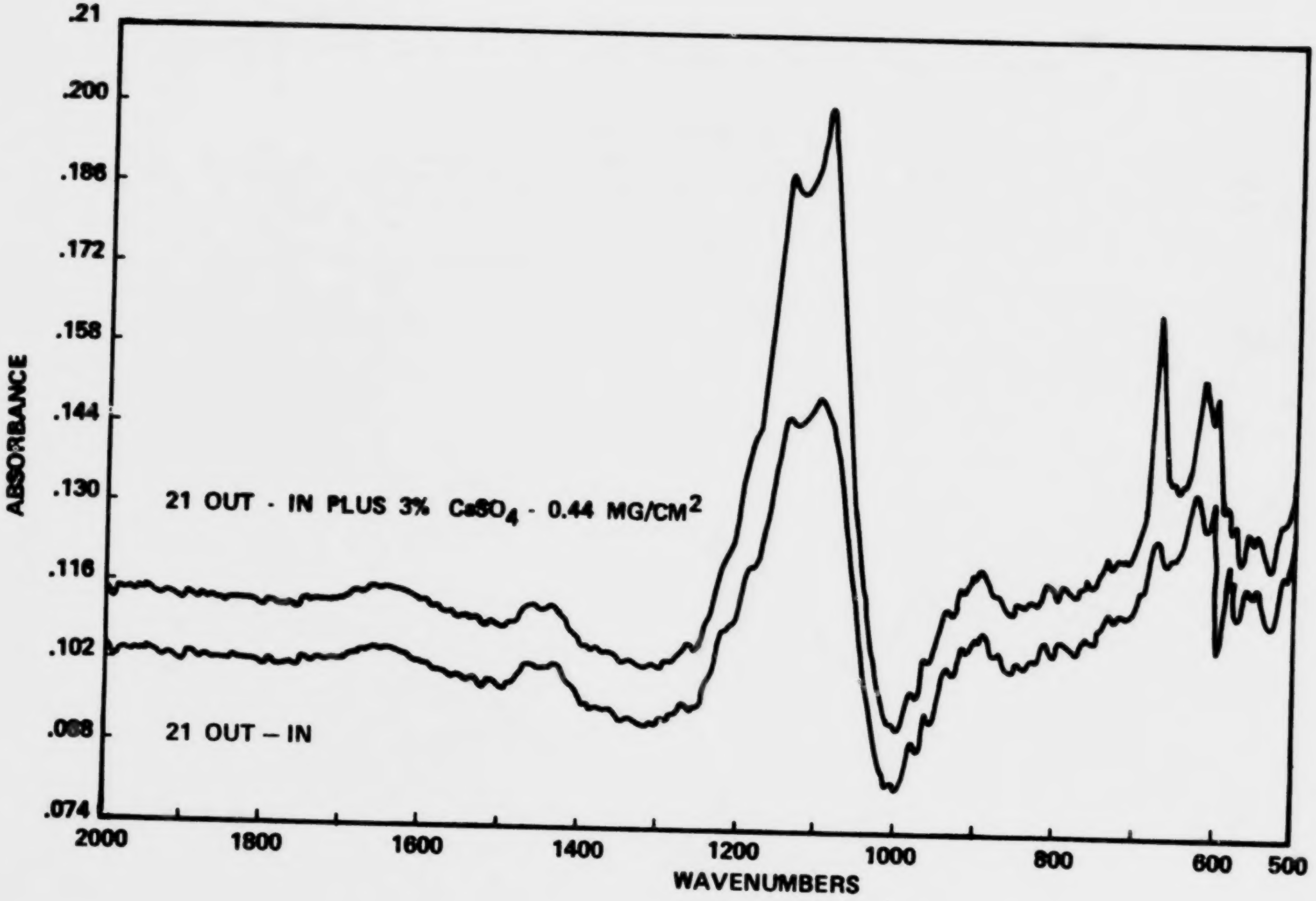


FIGURE 19 FTIR spectra showing the addition of 3% of the 0.4 mg/cm^2 $CaSO_4$ standard to the FGD difference spectra

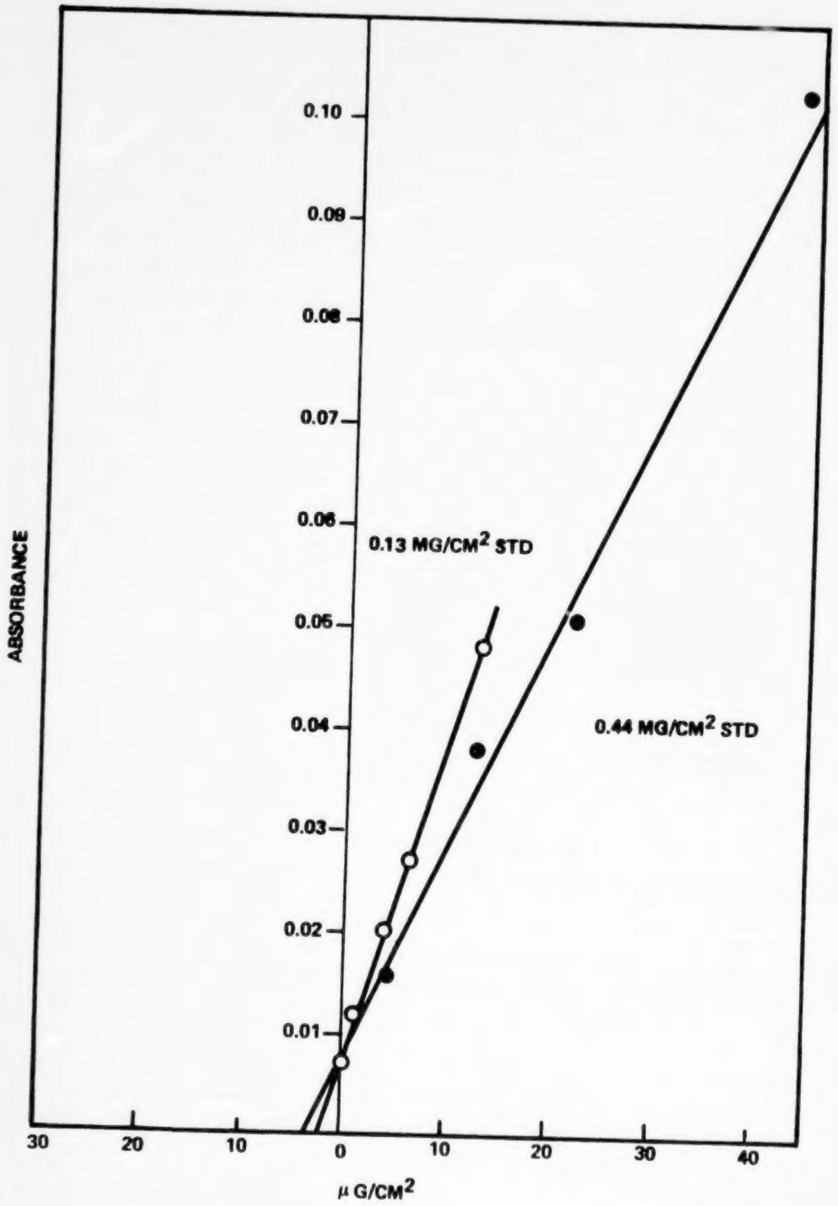


FIGURE 20 Standard addition curve for two different $CaSO_4$ standards

SUMMARY

The analysis of environmental samples is a complex task requiring the integration of various techniques. Starting with elemental analysis, information on the overall emission and composition of emitted particualte matter can be determined. It was found that certain elements (As, Cr, Mn, Ni, Sb, Se, V, and Zn) exhibit a concentration increase in the particulate matter emitted from the Firestone FGD system compared to the material entering a FGD under both oil and coal-firings. Impactor studies combined with elemental analysis have shown that this enrichment is due to the preferential emission of small particles (<3 μm) which contain increased concentrations of these trace elements. Surface analysis using ESCA showed that particles emitted from the La Cygne FaD system were coated with sulfur and carbon.

The materials emitted from an FGD can be identified using FTIR. FTIR provides all of the information of conventional IR and adds the ability to add, subtract, or ratio spectra to enhance differences or improve the sensitivity. Using $CaSO_4$ standards dispersed on glassfiber filters, FTIR spectra were obtained and used to electronically spike the sample spectra. $CaSO_4$ was identified in the outlet filter and quantitated. Sensitives on the order of 0.1 $\mu g/cm^2$ can be expected for FTIR.

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FTIR: A TOOL FOR BOTH ORGANIC AND INORGANIC ANALYSES IN ENVIRONMENTAL ASSESSMENT PROGRAMS

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ABSTRACT

Fourier Transform infrared spectroscopy (FTIR) can characterize both organic and inorganic species in a given sample. Application of this technique to environmental assessment programs and various sample handling methods are discussed. Two developing areas, inorganic compound speciation and on-the-fly GC/FTIR, are discussed in relation to their potential contributions to the field of environmental assessment.

INTRODUCTION

Fourier Transform infrared spectroscopy (FTIR) is a versatile tool in the field of environmental assessment because it is the only method by which both organic and inorganic compounds can be analyzed. With this capability, FTIR can be utilized in either overview-type programs such as Level 1 or can characterize samples compound by compound.

INORGANIC ANALYSIS

The ability to identify inorganic compounds in environmental samples places FTIR in a position of considerable importance. Currently, most inorganic analyses of samples for environmental assessment provide only elemental information, or at best, anion and cation concentrations. In rating the potential hazard of an effluent, this type of information is not sufficient; the form which an element or ion takes, to a great extent, determines the health effects it may have. This problem may be directly addressed by the use of FTIR for inorganic species information.

Figure 1 shows the spectrum of an ambient air particulate; in this case, one was collected by the National Bureau of Standards as their standard urban sample. This sample underwent no sample preparation whatsoever aside from grinding and mixing with potassium bromide (KBr) in order to produce a KBr pellet. In addition, only about 1 mg of sample is necessary to produce a spectrum. Interpretation of the spectrum reveals the presence of the following:

bound water	(3300-3600 cm^{-1})	carbonate	(1410-1450 cm^{-1})
	(1600-1650 cm^{-1})		
ammonium	(2800-3300 cm^{-1})		
hydrocarbon	(2800-3000 cm^{-1})	sulfate	(1090-1150 cm^{-1})
nitrate	(1380-1390 cm^{-1})	silicate	(1000-1080 cm^{-1})
		metal oxides	(400-600 cm^{-1})

Thus, in fewer than fifteen minutes from receipt of sample through interpretation of the spectrum, a good deal of information concerning the sample has been obtained.

Often programs endeavor to compare effluents. A single spectrum of the samples in question will aid such a comparison. Figure 2 shows the spectra of four ambient air particulates, from as-received samples, obtained in four cities.

Baton Rouge (the top spectrum) consists of almost pure ammonium sulfate. Upland (the bottom spectrum), on the other hand, has a low sulfate concentration (band near 1100 cm^{-1}), and the sulfate is not ammonium sulfate. In this sample, a relatively large amount of nitrate (1385 cm^{-1}) is present. Birmingham and Charleston both show significant levels of sulfates ($\sim$1100 cm^{-1}) and silicates ($\sim$1040 cm^{-1}) although in different ratios and varying types of sulfates. These two samples also contain carbonate ($\sim$1430 cm^{-1}) and nitrate (1385 cm^{-1}).

If comparative data are desired, a comparison of the spectra of the unfractionated samples is a very good place to start. If, however, more specific inorganic compound information is needed, we have had good success using a simple water extraction to separate species for analysis. Figure 3 shows the spectrum of the water insoluble portion of the NBS sample.

This spectrum indicates predominately silicates ($\sim$1040 cm^{-1}), probably complexed with metal oxides whose presence is indicated by bands below 600 cm^{-1}. In addition, from spectra of this fraction, we can obtain a relative measure of the crystallinity of the silicate by the intensity and splitting of the band near 800 cm^{-1}. Silicate and metal oxide species are seen in virtually all water insoluble fractions of environmental samples.

The real utility of this water extraction is in removing the above mentioned species so that we can isolate the water solubles, which typically contain such species as nitrates, sulfates and carbonates. In particular, the spectrum of the NBS sample (see Figure 4) indicates OH^-, NH_4^+, $CO_3^=$ and NO_3^- functionalities. From this spectrum we can positively identify $CaSO_4$ as the major component of this fraction by the position of the S-O stretching vibrations (1100-1150 cm^{-1}) and by the more characteristic position of the S-O bending bands between 600 and 700 cm^{-1}. We also know from the spectrum that $CaSO_4$ is present as the dihydrate by the shape and position of the OH stretching and bending bands near 3500 and 1600 cm^{-1}.

Another sample handling method that we have found helpful is the splitting of a sample or fraction and heating one-half at 200°C for 3 hours, leaving the other half unbaked. This procedure both "cleans up" the spectrum by removing volatiles and extraneous water and also for many compounds will cause them to assume a reproducible hydration state. The spectral changes, which occur with hydration state changes, tend to be reproducible and quite informative. In Figure 5 are two spectra: the lower is the methanol extract residue of the as-received NBS; the upper is the methanol extract residue of a portion of the NBS sample which had been baked as described above. The methanol extraction was originally performed in order to isolate the more polar organics. While organic material is certainly present in the unbaked sample (bands near 2900 cm^{-1}), the number and broadness of bands below 1700 cm^{-1} suggests a substantial inorganic content and sorting out inorganic versus organic species in this setting is difficult. The spectrum of baked residue shows almost pure ammonium sulfate. This accounts for all of the sulfate but only part of the nitrate and parts of the other two major bands. Now, with knowledge of the polarity of the solvent, the remaining bands can be assigned to short chain carboxylic acid and ammonium nitrate. Ammonium chloride may also be present.

In the past, it was thought that interactions of sample with potassium bromide made the pellet technique a poor one. While interactions do occur with some substances, we have found that the KBr pellet produces very good results with most inorganics and that in fact, on our instrument, frequency reproducibility is excellent (1).

To illustrate this reproducibility, we had several people prepare pellets of $CaSO_4$ in KBr over a two-week period and ran spectra on two FTIR systems. We chose KBr pellets for this experiment because this is how we most commonly run environmental samples. The results are summarized in Table 1. In all three of the band positions shown, reproducibility was within 2 cm^{-1}.

Using this precision, we have created our own computerized spectral search system. Sulfates, carbonates and nitrates have been run as received as well as after dissolution in water and heating. That is, the reference compounds are treated in the same manner as the samples will be. Three parameters are entered into the system for each band in both reference and sample spectra: band position (frequency), band intensity and band width at half height. In addition, each reference band is assigned a priority, thus utilizing the skill of the spectroscopist.

Figure 6 shows the results of the spectral search for the spectrum of the unbaked water soluble fraction which we saw previously. Note that the compound given the highest ranking (that is, considered most likely to be present) is $CaSO_4$ which we have already identified as the major component of this fraction. Note that all compounds are designated as either U or B denoting either the unbaked or baked condition.

The real power of this spectral search is shown in looking at more minor components. For instance, $ZnSO_4$ is listed in the eighth most likely position, but is it present in the sample or not? First, we note that in the unbaked sample, the computer sees unbaked $ZnSO_4$. Below, the bands used to achieve the match are summarized. On the computer match, this looks very good, but referring back to the actual spectrum (Figure 4), we see that in the complex mixture of S-O stretching and S-O bending bands the match is hardly conclusive. For corroborating evidence, we look at the results of the spectral search on the spectrum of the baked water soluble fraction (Figure 7). If $ZnSO_4$ is present, a match should now be made with $ZnSO_4$ in the baked condition and indeed this match is seen in Figure 7. The match is more convincing because band positions of the baked sample have shifted as compared to the unbaked sample (as shown in the lower section of Figure 7), and still match. The presence of $ZnSO_4$ is further supported by elemental analysis indicating both Zn and $SO_4^{=}$ in this fraction. Thus, the computer assisted spectral interpretation program can greatly speed the interpretation of the spectra.

We have also found that for many inorganics pelleting in KBr produces spectral bands whose intensities are linear with concentration. Figure 8 plots three bands of Li_2CO_3 in intensity versus concentration in KBr. All three band intensities were found to be quite linear with concentration, certainly well within our gravimetric error. Not only does this raise the possibility of quantitation of inorganics at the source, but the ease of obtaining data is truly impressive; all pellets were prepared, spectra obtained and the graph produced by the computer in the period of about one hour.

ORGANIC ANALYSIS

So far, only inorganic compounds have been considered, yet organics are an important part of the sample and are more traditionally the province of infrared spectroscopy. Easily extracted from the bulk of the sample, organics can be analyzed separately, leaving the inorganics for separate treatment. After extraction, a fast, efficient method of obtaining class information is the Level 1 liquid chromatography separation of the sample into seven fractions of increasing polarity, after which infrared spectra are obtained of each fraction. One of the classic problems of this method, however, is the difficulty in obtaining clean separations; that is, there can be a great deal of overlap between fractions, making interpretation of spectra more difficult. An example of this is seen in Figure 9. The top spectrum is fraction 3 from a typical Level 1 SASS train sample. There is aliphatic hydrocarbon seen in conjunction with what could be two carbonyls and some aromaticity, but it is impossible to assign which bands go with which, or even make more than a rough guess at how many compounds are present. Fraction 2, seen at the bottom of the figure, can easily be identified as an ester. Comparing the two spectra, fraction 3 appears to possibly contain fraction 2. A subfraction (the middle spectrum) reveals that not only is the ester a constituent of fraction 3, but that on digital removal of the ester, a quinone can quite easily be identified as the other compound present in this fraction.

As with inorganics, a cleaner separation is needed for compound identifications to be made. In the case of organics, the low concentrations of materials may cause problems due to detection limits. Isolation of these minor components may also be difficult, however, two techniques of promise are HPLC/FTIR and GC/FTIR. We are developing GC/FTIR in our laboratories and GC/MS is already widely used in environmental assessment. Since both of these techniques are useful in some settings, we decided to compare the two (2). The three points to be considered are:

(1) sensitivity
(2) speed and ease of operation
(3) amount of information

Figure 10 shows a gas chromatogram of a wastewater sample run on a packed column with a flame ionization detector (FID). Although this particular work was done on packed columns, we have used both wall-coated and support-coated capillary columns on line with the FTIR system. Each number represents an infrared spectrum obtained at that position. During the most intense peak (aside from the solvent peak), three spectra were taken: numbers 2, 3 and 4, corresponding to the leading edge, center and trailing edge of the peak. Figure 11 shows these three spectra.

The spectra are seen to be of quite good quality, and they are quite distinct, representing the para, meta and ortho isomers of chloronitrobenzene. This ability of FTIR to isolate isomers, which GC/MS cannot do, has important applications in environmental assessment, as quite often only one of several isomers is toxic. In addition, IR can provide functional group information which is useful even if the exact molecule cannot be identified. This also is a useful advantage of GC/IR over GC/MS.

Table 2 summarizes results obtained for this sample by the two techniques: GC/FTIR and GC/MS. Obviously, the two are complementary; on the one hand, GC/IR can give isomer information that MS cannot. On the other hand, GC/MS can elucidate compound structure where GC/IR gives only functional group and class type information such as dibutyl phthalate versus alkyl phthalate. Some of the GC/FTIR identifications are marked as tentative. This is because, as yet, the library of vapor phase infrared spectra is limited to several thousand compounds and identifications must often be made using condensed phase reference spectra which may be drastically different from spectra in the vapor phase. Here GC/MS has a great advantage, at least temporarily. Their reference library exceeds that of GC/IR by more than a factor of 10 and computer software available for GC/MS is currently more advanced that that available for GC/FTIR.

Returning to the three points of comparison, we can see that

(1) the quality of information from the two techniques is very complementary in nature,

(2) GC/MS speed and ease of operation is much better than that of GC/FTIR for the time being, and

(3) GC/MS theoretically has a greater potential sensitivity. However, in complex samples, sensitivity is often matrix dependent and GC/FTIR under certain conditions will have equal or better sensitivity than GC/MS.

SUMMARY

In conclusion, the versatility of FTIR makes possible a variety of sample handling techniques. Information concerning both inorganics and organics can range from simple class determination to compound by compound characterization of the sample. Methods currently in developmental stages, such as the computer assisted spectral interpretation program and GC/FTIR, promise rapid expansion of application of FTIR to the field of environmental assessment.

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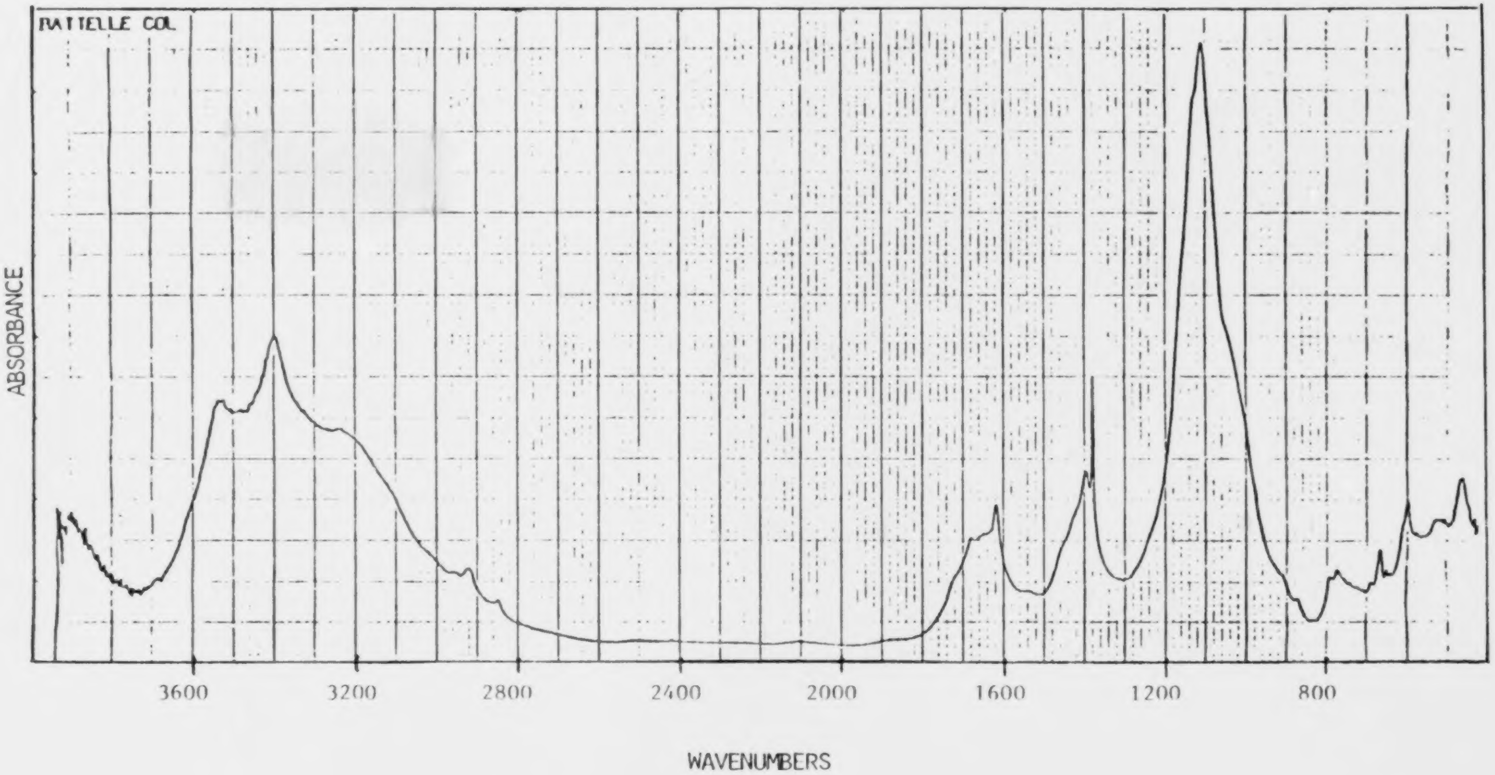


FIGURE 1 IR spectrum of an ambient air particulate sample

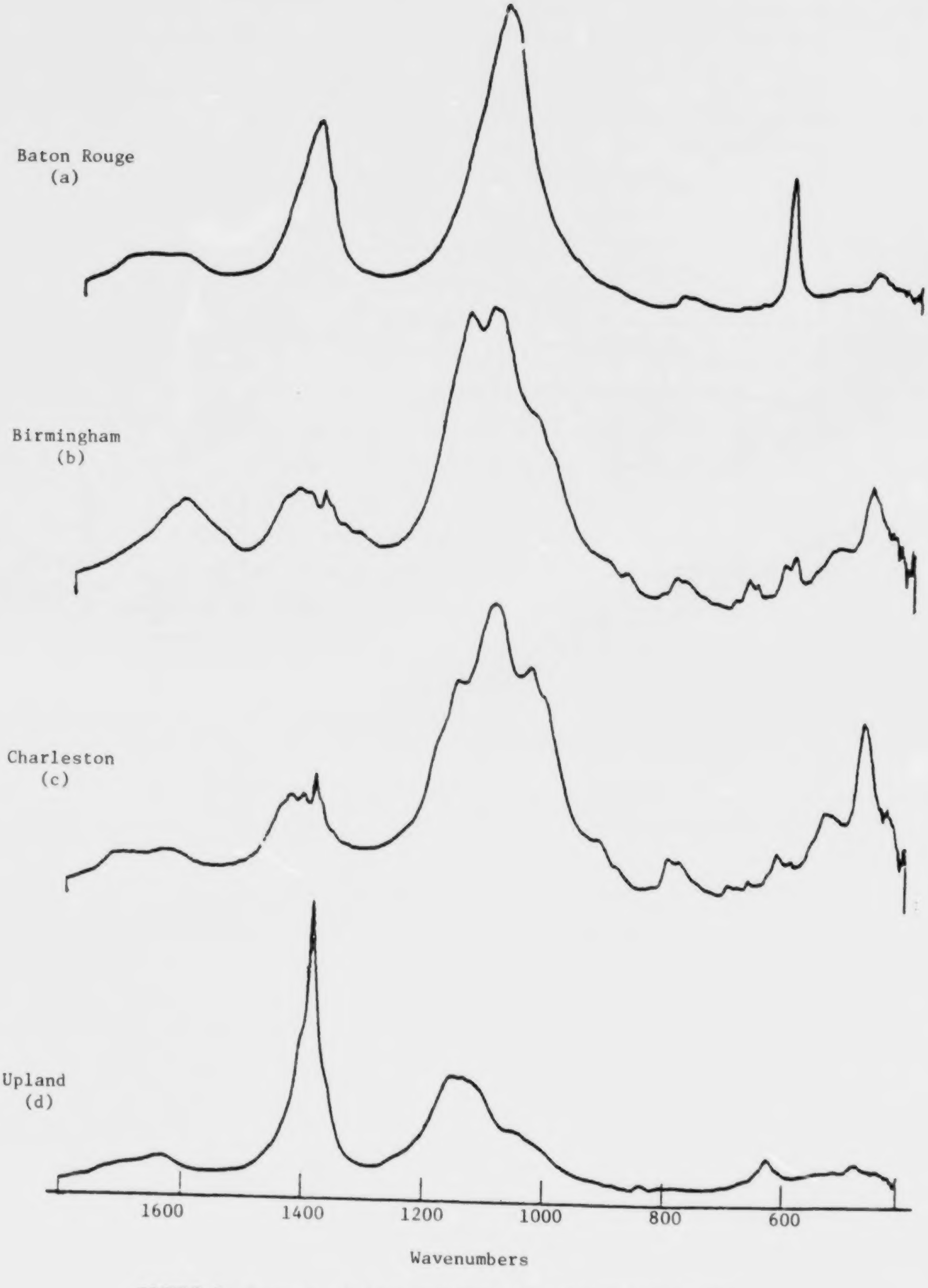


FIGURE 2 Spectra of four ambient air particulate samples

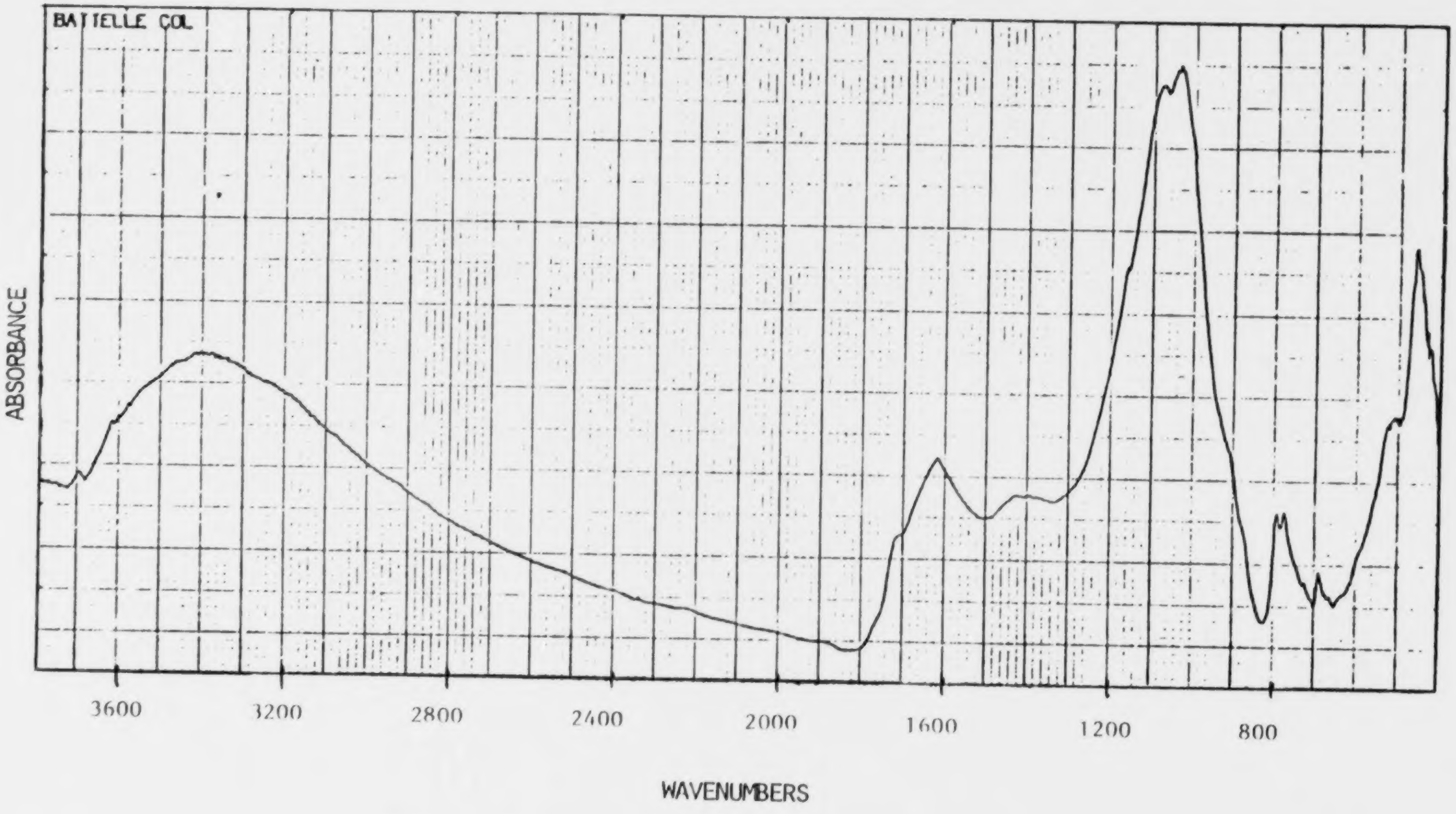


FIGURE 3 IR spectrum of the water insoluble portion of an ambient air particulate sample

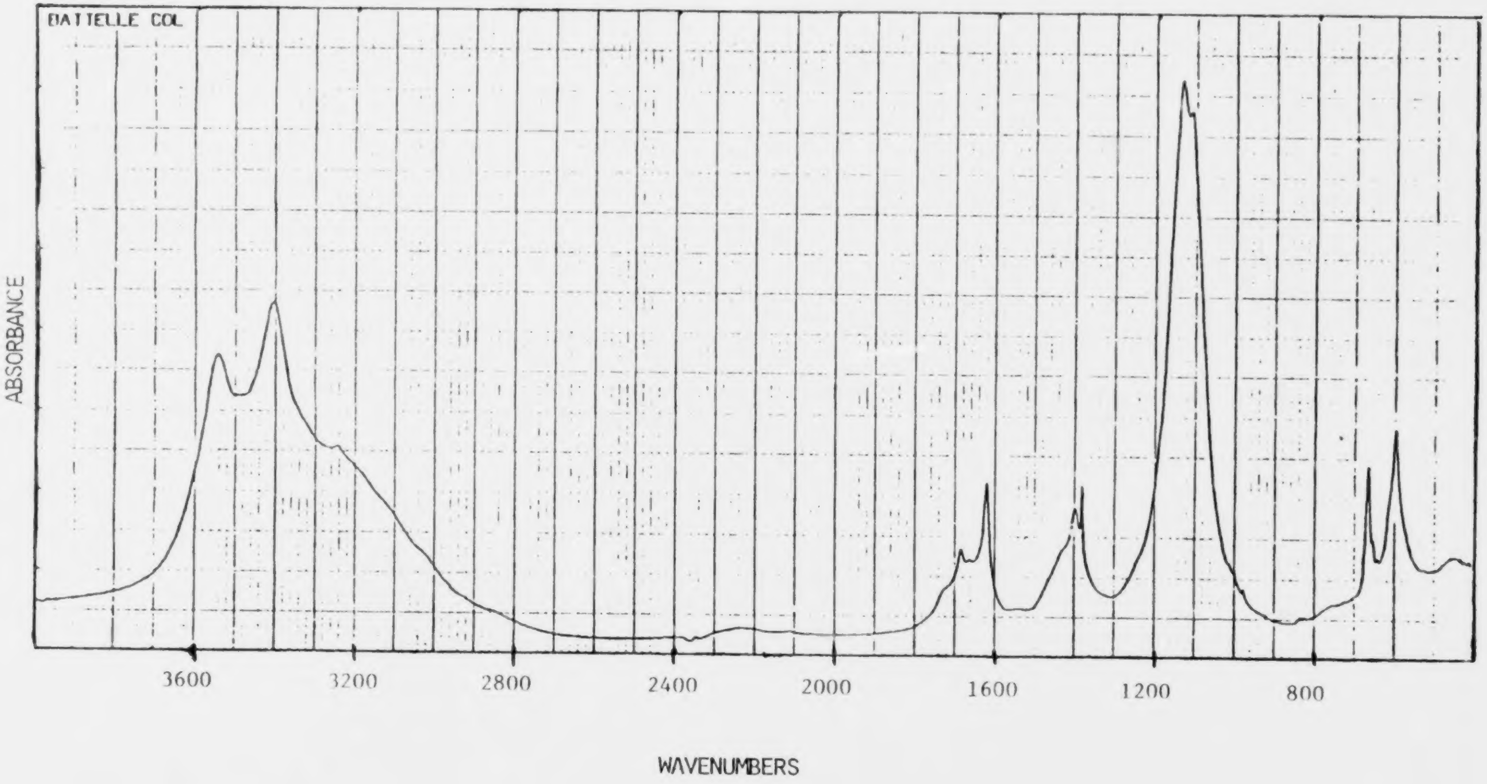


FIGURE 4 IR spectrum of the water soluble portion of an ambient air particulate sample

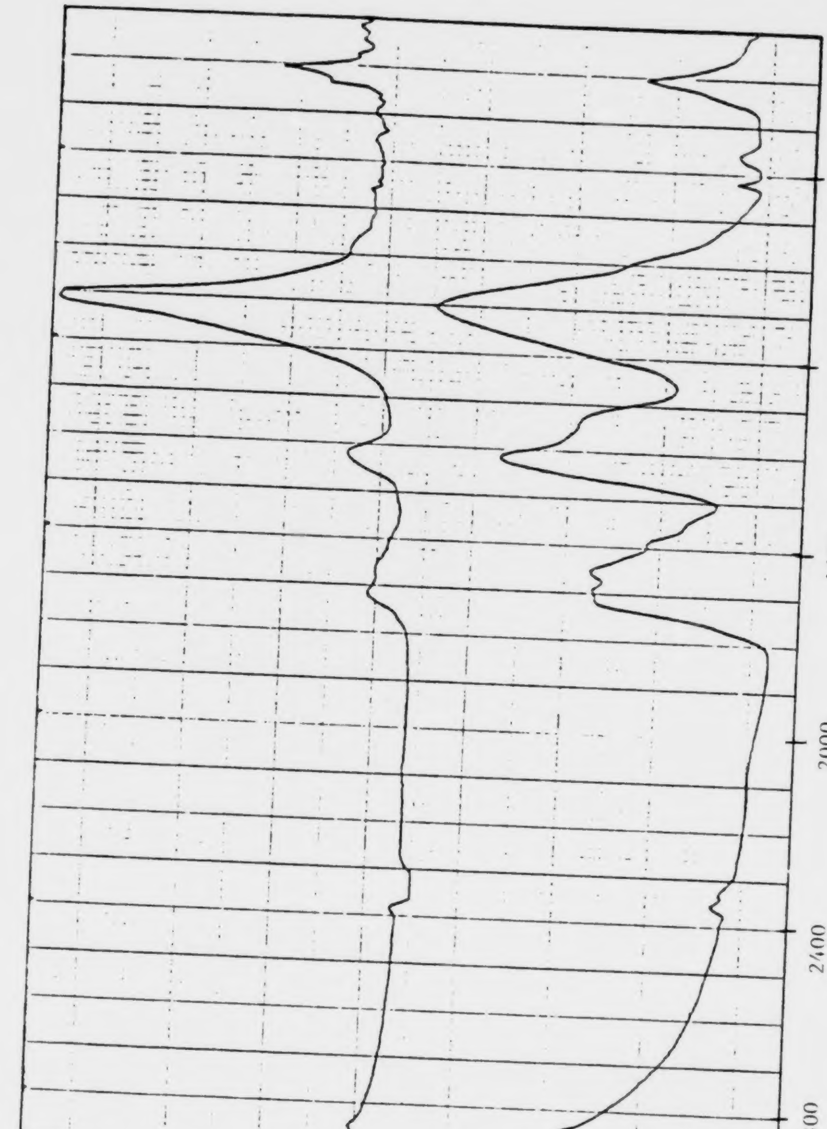


FIGURE 5 IR spectra of methanol extracts of an ambient air particulate sample (upper curve for sample baked at 200°C, 3 hrs; lower curve for sample as received).

MATCHING FILE--UR34UUU
THE BEST MATCHES WERE AS FOLLOWS

REFERENCE FILE	SCORE	BASE SCORE	MATCHES	RANK
$CaSO_4$-U	417	225	7	1
$FeNH_4(SO_4)_2$	281	126	3	2
$AlNH_4(SO_4)_2$-B	279	102	4	3
NH_4NO_3	210	104	4	4
$(NH_4)_2SO_4$	202	66	2	5
Na_2SO_4	165	59	3	6
$Fe_2(SO_4)_3$-B	154	73	4	7
$ZnSO_4$-U	152	62	2	8
$MnSO_4$-U	149	92	5	9
$CaSO_4$-B	140	64	5	10

REFERENCE FREQ.	SAMPLE FREQ.	REF. INTEN.	REF. PRIOR.
$ZnSO_4$-U			
1142	1138	3.0	4.0
613	602	1.0	2.0

#34 H_2O Soluble Unbaked

* B=Baked sample, U=Unbaked sample

FIGURE 6 Results of the spectral search for the spectrum of the unbaked water soluble fraction.

TABLE 1

Spectra of $CaSO_4$ Pellets Prepared in KBr

$CaSO_4 \cdot 2H_2O$

Date Run	Instrument	Band 1	Band 2	Band 3
4/10	FTS-10	1622.2	668.4	601.8
4/13	FTS-10	1266.2	666.5	599.9
4/15	FTS-10	1624.2	668.4	599.9
4/17	FTS-10	1621.3	669.3	598.9
4/23	FTS-10	1621.3	668.4	600.9
5/2	FTS-10	1621.3	668.0	600.9
5/3	FTS-10	1622.2	668.0	600.9
5/4	FTS-10	1621.3	668.4	600.9
4/17	FTS-14	1621.3	668.4	600.9
4/20	FTS-14	1621.3	668.4	599.9
4/23	FTS-14	1621.3	668.4	602.8
4/24	FTS-14	1621.3	668.4	601.8
4/25	FTS-14	1621.3	668.4	602.8
4/25	FTS-14	1621.3	668.4	602.8

MATCHING FILE--URA21
THE BEST MATCHES WERE AS FOLLOWS

REFEREBCE FILE	SCORE	BASE SCORE	MATCHES	RANK
$CaSO_4$-B*	285	146	7	1
$MgSO_4$-U	222	93	4	2
$MnSO_4$-U	202	110	5	3
$MgSO_4$-B	192	75	5	4
$ZnSO_4$-U	189	92	5	5
$ZnSO_4$-B	180	80	4	6
$Alk(SO_4)_2$-U	179	79	3	7
Na_2SO_4-U	172	68	3	8

REFERENCE FREQ.	SAMP. FREQ.	REF. INTEN.	REF. PRIOR.
$ZaSO_4$-B			
1142	1152	3.0	3.0
1101	1115	3.0	4.0
1101	1098	3.0	4.0
631	629	2.0	3.0

#21 H_2O Soluble baked

* B=Baked sample, U=Unbaked sample

FIGURE 7 Results of the spectral search of a baked water soluble fraction.

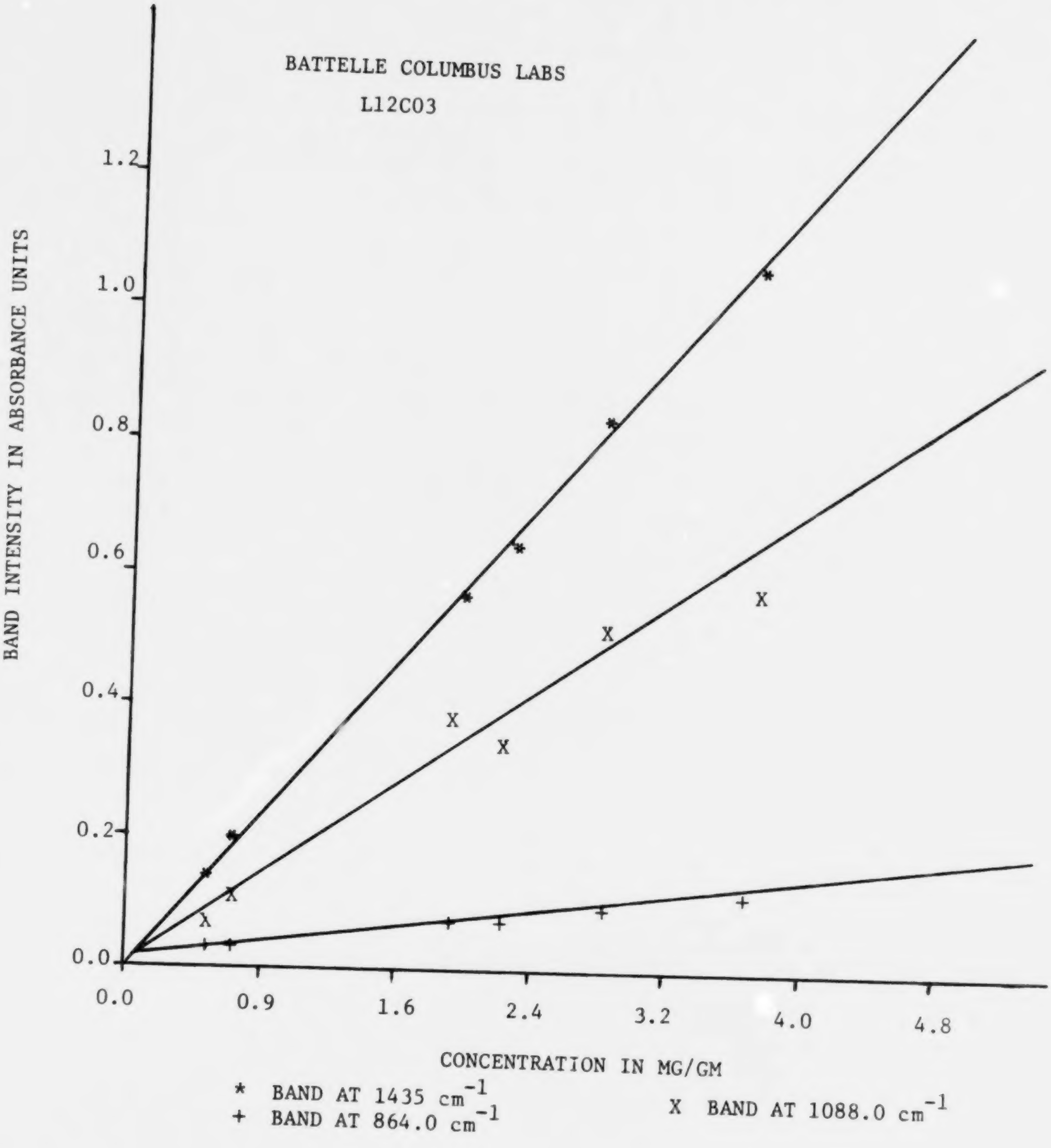


FIGURE 8 Intensity versus concentration of Li_2CO_3 in KBr.

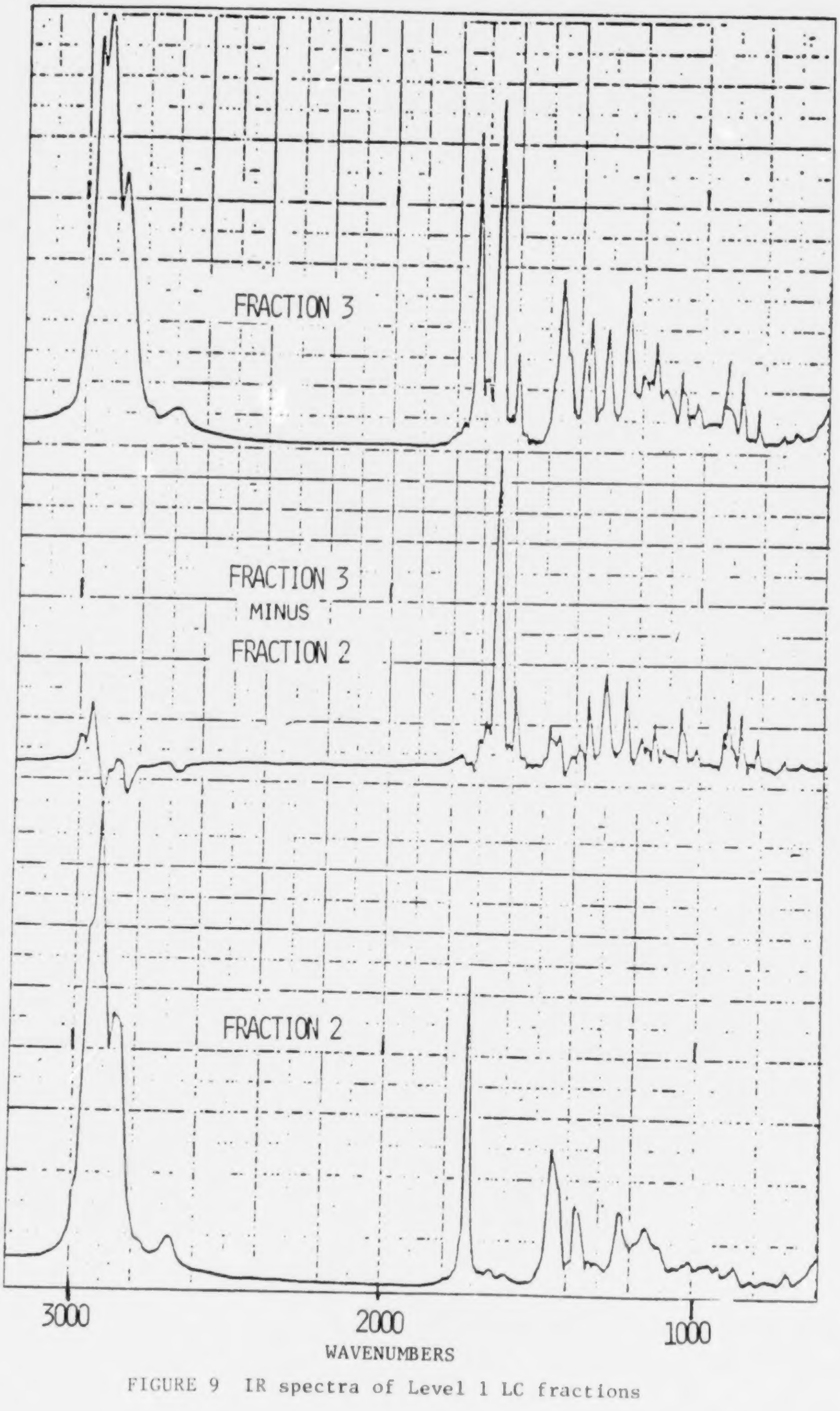


FIGURE 9 IR spectra of Level 1 LC fractions

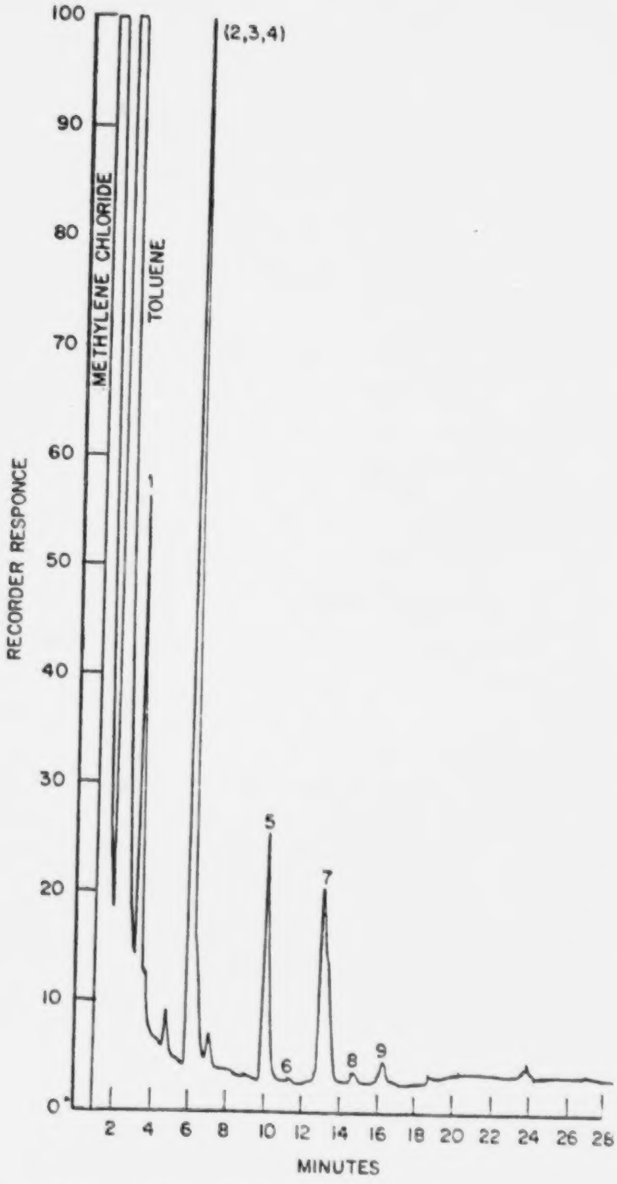
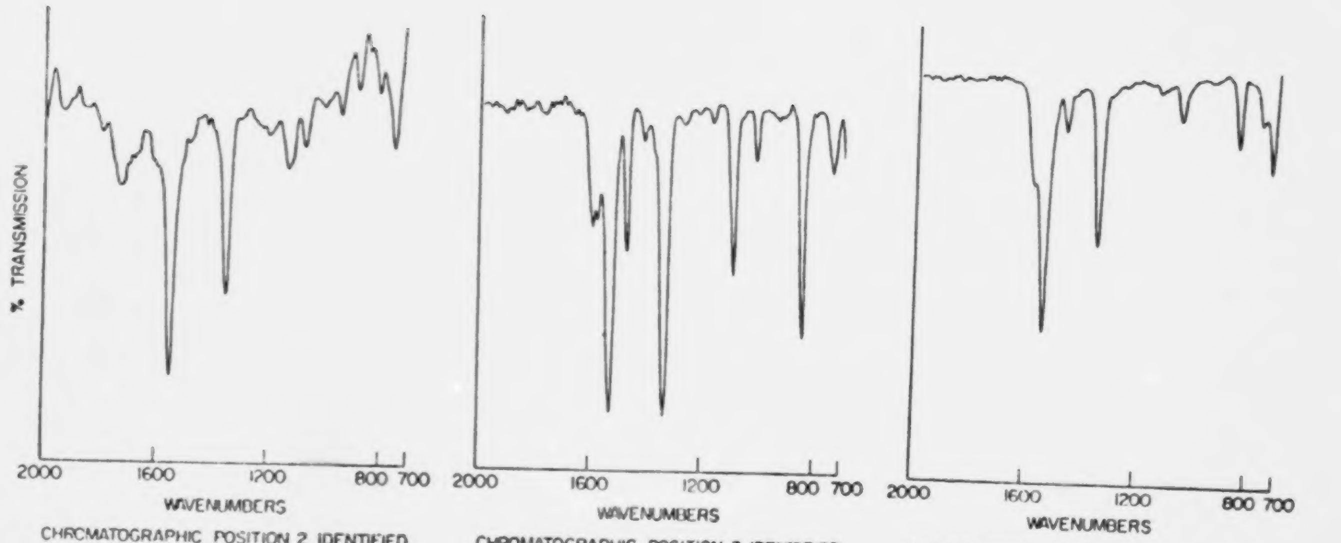


FIGURE 10 Chromatogram of GC separation



CHRCMATOGRAPHIC POSITION 2 IDENTIFIED AS m-CHLORONITROBENZENE

CHROMATOGRAPHIC POSITION 3 IDENTIFIED AS p-CHLOROITROBENZENE

CHROMATOGRAPHIC POSITION 4 IDENTIFIED AS o-CHLORONITROBENZENE

FIGURE 11 Successive IR spectra obtained during elution of a gas chromatographic peak.

TABLE 2

Results of GC/FTIR and GC/MS Analysis

GC/FTIR Identifications	GC/MS Identifications
p-Dichlorobenzene	
m-Chloronitrobenzene	
p-Chloronitrobenzene	Isomers of chloronitrobenzene
o-Chloronitrobenzene	
3,4-Dichloronitrobenzene	Dichloronitrobenzene
Tentative identification of 2,4-dichloro-6-nitroaniline	Dichloronitroaniline
Tentative identification of either trichloronitrobenzene or 2,3-dichloronitroaniline	Trichloronitrobenzene
Alkyl phthalate	Dibutyl phthalate Dibenzyl or butylbenzyl phthalate
Tentative identification of triphenylphosphate	Triphenylphosphate
	Tentative identification of abietic acid

PB82-211897

MEASUREMENT METHODS FOR SO_x AND NO_x IN THE PRESENCE OF NH_3

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Technology Division

Ammonia reacts with SO_x and NO_x to form various ammonium sulfur and nitrogen compounds. These compounds can precipitate in standard sampling systems and obstruct the flow of sample gas yielding invalid results. Moreover, most of the analytical procedures used in conjunction with SO_x, NO_x and NH_3 Standard Method determinations are adversely affected when all three of these components are present in the gas stream. This paper presents the results of a series of laboratory studies in which modifications to both the sampling systems and the analytical procedures were made in order to adequately measure these parameters.

Various levels of ammonia were injected into a sample stream containing typical stock concentrations of SO_x, NO_x and water vapor. This sample stream was used to determine the effects of NH_3 on the Methods. Sampling train modifications including precipitate traps, NH_3 and SO_3 scrubbers were implemented. Alternate analytical procedures compatible with these complex stream were developed.

PB82-211798

EVALUATION OF STABLE LABELED COMPOUNDS AS INTERNAL STANDARDS FOR QUANTITATIVE GC/MS

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Systems, Science and Software

Quantitation of organic compounds by GC/MS using stable labeled compounds as internal standards is a widely accepted method of preference in many research areas. The reasons for this are all related to the *in situ* recovery compensation which is built into each analysis and the resulting high accuracy which this provides. These analyses have typically been empirically optimized for a single compound of interest and, although the technique is simple, attempts to expand the approach for multiple component applications have been very limited. The reasons generally expressed for not expanding this technology to the multiple component analysis generally encountered in process measurement studies for environmental assessments, revolve around potential high costs and poor availability of labeled materials, possible problems with label exchange, difficulty in identifying proper experimental parameters, variable label purity of commercially available compounds and propagation of errors during data reduction. Each of these potential problem areas has been investigated in detail for a selected group of compounds, the organic priority pollutants, and the results indicate that an expansion of this methodology to certain types of environmental assessment measurements could lead to improved analytical results at reduced cost. The results of these studies will be given in this presentation.

LEVEL 1 ENVIRONMENTAL ASSESSMENT: FLUIDIZED-BED COMBUSTION

R. R. Hall, P. F. Fennelly,
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Technology Division

Environmental assessment samples were collected at the EPA-sponsored Exxon Miniplant, a pressurized fluidized-bed combustor, in May 1979. Level 1 chemical and biological tests have been completed and results are presented.

Improved particulate control has almost eliminated potential trace element emission problems. Level 2 tests that will focus on positive mutagenicity results in the Ames *Salmonella* Microsome assay are outlined.

PB82-211806

EVALUATION OF LEVEL 1 ANALYSIS PROCEDURES

R. K. M. Jayanty, W. F. Gutknecht,
A. Gaskill, Jr. and D. E. Lentzen
Research Triangle Institute

The current environmental assessment (EA) program of the Industrial Environmental Research Laboratory, Research Triangle Park (IERL-RTP), is designed to yield data that will result in the identification of sources of recognized pollutants and other substances of potential environmental concern. As a part of the IERL-RTP Quality Assurance Program for assessing data quality, two components of the EPA process--the analytical methods and their application--were evaluated. Eight contractors were supplied with four simulated organic and inorganic source assessment samples for analysis. The four audit samples were (1) a five-component synthetic organic mixture chosen to evaluate the Level 1 organic measurement techniques of IR, LRMS, TCO, GRAV, and LC; (2) a commercial dye mixture selected to test the LC scheme and to be tested as a general procedure for assessing the performance of the Level 1 LC column; (3) a modified fly ash sample selected to test the SSMS and cold vapor atomic absorption techniques; and (4) a clean blank XAD-2 resin selected to check the completeness of Parr bomb ashing of the resin and contamination of the sample during the ashing process.

The specific objectives of this audit program and the results reported by the contractors will be presented. Problems experienced by the participating contractors for both organic and inorganic analyses using Level 1 analytical techniques will be discussed. In general, the problems identified with organic analyses are data reduction errors, loss of volatile substances, incomplete drying for GRAV analysis, and contamination or misinterpretation of spectra. Several problems identified with SSMS, including incorrect reporting of results, quantitation of detection limit values, and low recoveries for Be and Cd, will be discussed. The accuracy, precision, and overall acceptability of the Level 1 analysis procedures will be addressed and recommendations for improving the data quality will be presented.

ANOMALOUS HIGH TOTAL CYANIDE RESULTS DUE TO NITRITE IN BIOTREATER EFFLUENTS: THE KEY TO THE "CYANIDE-GENERATION SYNDROME" IN BIOTREATER TECHNOLOGY

R. A. Johnson, J. C. Rapean
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Shell Development Co.

The Total Cyanide Method, involving the distillation of HCN from dilute sulfuric acid, is notorious for yielding erratic results. Even in laboratories with the method "in control", sporadic excursions occur in an unexplained way. This behavior is especially troublesome in the determination of cyanide in feeds to and effluents from industrial wastewater biotreaters. These Total Cyanide results take on an extra dimension of importance when they are involved in NPDES reporting.

In biotreater technology, a "cyanide-generation syndrome" is sometimes observed in which the Total Cyanide measured in the effluent greatly exceeds that in the feed and results in NPDES permit violations. The explanation of the sporadic apparent generation of cyanide in certain biotreaters has weighed heavily on the concerned analytical laboratories and biotreater operators.

The key to this problem is now being found in the anomalous high results for Total Cyanide due to the reaction of nitrous acid with certain organic compounds to produce HCN. The nitrous acid is variably produced by microbial nitrification in biotreaters. The variations in nitrite in the samples parallel the variations in the Total Cyanide found. This analytical anomaly is avoided by the reduction of nitrous acid with sulfamic acid prior to the distillation step. This effect was observed and described by S. Kanno and co-workers in Japanese journals in 1974 and 1975[1] and, in English, in Chemosphere[2] more recently. However, this anomaly has apparently remained unobserved in this country until recently. Acting on Kanno's work, Shell Development Company modified its Total Cyanide Method for wastewater and was able to solve the mystery of the "Cyanide-Generation Syndrome" in a biotreater at one of the Shell plants.

Independently, Jeremiah Casey and co-workers of Air Products Co.,[3] rediscovered the effect and solved a similar problem.

This poster session is provided to spread the understanding of this solution to a perplexing problem in the critical area of cyanide discharges in treated wastewaters.

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3. J. Casey et al., to be published in Analytical Chem. in 1980.

DESIGN MODIFICATIONS TO THE SOURCE ASSESSMENT SAMPLING SYSTEM

R. L. Campbell, K. W. Mason,
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PEDCo Environmental, Inc.

Recently, there has been an interest in improving the Source Assessment Sampling System (SASS) from a user and performance standpoint. Based on earlier designs, a complete SASS train was designed and built with major modifications incorporated. These major modifications include a larger filter holder, separation of the filter and cyclones into separate ovens, a complete glass organic module, using a refrigeration unit to control the sample gas temperature, and larger impingers equipped with all Teflon stems and connections. Field use of the train indicates significant improvements in operation. However, no comparative analytical data are available at this time. Recommendations for further design considerations are made.

DIAGNOSIS OF METAL SPECIATION IN AQUEOUS SOLUTIONS*

B. McDuffie and P. Figura**
State University of New York at Binghamton

A method for differentiating soluble trace metal species on the basis of relative lability has been developed for solutions of Cd, Cu, Pb and Zn utilizing anodic stripping voltammetry (ASV) and Ca-Chelex resin in successive column and batch procedures. Species are classified as being "very labile", "moderately labile", "slowly labile" or "inert", depending on the characteristic time scale of the measuring technique. Although ASV has limitations, most heavy metals can be put into categories based on Chelex uptake. A range of dissociation rate constants can be estimated for metal complexes in each category. Preconcentration of the various fractions leads to precise determinations even at ppb levels. The method has been applied to water from the St. Lawrence and Susquehanna Rivers, to a Hudson River estuary sample, and to secondary sewage effluent. In spite of the variety of samples, reasonable distinctive patterns of speciation were observed. Cadmium and zinc occurred almost entirely in the "very labile" and "moderately labile" fractions. Copper was found primarily in the "moderately labile" and "slowly labile" fractions, no "very labile" copper being found. Lead showed some "very labile" species, 20-70% "slowly labile" material, and a significant "inert" fraction in several cases. Factors related to application of this method to process measurements will be explored.

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*Work supported in part by USDI - OWRT Matching Grant B-070-NY.

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PB82-211848

SOLID SORBENTS FOR AIR SAMPLING

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This paper gives results of an ongoing experimental program to evaluate the breakthrough characteristics of sorbent resins for sampling of organic vapors using an elution analysis chromatographic technique. The effects of water vapor and CO_2, at levels typical of gaseous effluents from combustion processes, on retention of nonpolar and polar species on two commonly used sorbents (XAD-2 and Tenax-GC) have been studied. Effects on XAD-2 were small but the volumetric capacity of Tenax-GC was substantially decreased. Other sorbents were characterized for potential use in vapor sampling systems: coconut-based charcoal, petroleum-based charcoal, silica gel, Ambersorb XE-340 and XE-347, and XAD-8. Retention volumes for XAD-8 and silica gel were roughly comparable to those of XAD-2 and Tenax-GC; those of the charcoals and the Ambersorbs were 2 to 4 orders of magnitude higher. Recovery of sorbate from charcoals is known to be poor in some cases, however, and recovery from the Ambersorbs is suspect but under investigation.

SCREENING - BIOLOGICAL AND CHEMICAL DATA ANALYSIS

N. H. Sexton and L. I. Southerland
Research Triangle Institute

The Industrial Environmental Research Laboratory (IERL) has developed a scheme for the assessment of the environmental hazard of effluents and emissions from industrial sources. This assessment scheme is composed of a screening step (Level 1), a detailed, quantitative analysis step (Level 2), and a long-term monitoring step (Level 3). This presentation deals with Level 1 which incorporates a battery of bioassays developed in a cooperative program with EPA Health and Ecological Laboratories, and a group of chemical analyses. These bioassays examine the health and ecological effects of varying levels of chemical by-products from four Level 1 studies. Three Level 1 pilot studies involved a coal gasifier, a fluidized-bed combustor, and a number of different aqueous textile plants. The fourth study involved a variety of industrial sources.

The basic approach for statistical analysis was to quantify, on a continuous or discrete (1,2,...) scale, the possible biological responses, and then to correlate the biological results with the amount of chemical present and correlate biological measures with other biological measures.

PB82-211863

ANALYSIS OF COAL LIQUID SUBFRACTIONS WHICH EXHIBIT MICROBIAL MUTAGENIC ACTIVITY

B. W. Wilson, R. A. Pelroy,
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Battelle Pacific Northwest Laboratory

The chemical composition and biological activity of a coal liquefaction material was compared before and after catalytic hydrogenation. Chemical analysis has employed solvent fractionation, gas chromatography/mass spectrometry and high pressure liquid chromatography and has been complemented by Ames assay for microbial mutagenic activity. In contrast to the predominately aromatic nature of the principal components of the coal oil, the hydrogenated products contain mainly hydroaromatic species and a significantly smaller fraction of basic and tar components. The microbial mutagenic activity of moderately and severely hydrotreated projects was reduced substantially to 2.8 percent and 0.8 percent, respectively, when normalized to that of the coal oil feed. These changes in biological activity have been related directly to the content of primary aromatic amines in the individual coal liquefaction materials.

PB82-211871

QUANTITATION OF POLYCYCLIC AROMATIC HYDROCARBONS IN COMPLEX MIXTURES BY HIGH RESOLUTION GLASS CAPILLARY GAS CHROMATOGRAPHY/MASS SPECTROMETRY USING SELECTED ION MONITORING

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U.S. Department of Energy
Pittsburgh Energy Technology Center

Quantitation of individual polycyclic aromatic hydrocarbon isomers in complex mixtures is a difficult task. Previous quantitation schemes have involved time consuming, manpower intensive preseparation of samples to produce an aromatic fraction which was subsequently quantitatively analyzed by capillary gas chromatography or capillary GC/MS using external or internal standards. These methods require the use of standards not present in the original sample and a knowledge of the relative response factors of the compounds of interest and the internal standards. One frequently neglected problem with this approach is that these relative response factors can change as a function of sample injection technique and the adsorptive characteristics of the chromatographic column. Thus, these methods often lead to quantitative results of poor precision and accuracy.

We will describe the application of the method of standard additions to quantitative PAH analysis by high resolution glass capillary gas chromatography/mass spectrometry and selection ion monitoring in samples where no preseparation has been performed. This technique provides quantitative values for PAH of high precision and accuracy even at the ppb concentration.

PB82-211889

ANALYTICAL RESULTS OF A PCB TEST INCINERATION

C. D. Wolbach, W. F. Fitch,
N. Flynn and B. Markoja
Acurex Corporation

Results of a test incineration of PCB-containing waste material are presented. A discussion of the analytical problems in quantitating PCB-containing samples is given with emphasis of the DCB methodology of armour. A listing of other identified species of environmental significance is offered.

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